2012

Population and conservation genetics of a habitat-specific riverine fish species, the eastern sand darter (Ammocrypta pellucida).

Robert Ginson
University of Windsor

POPULATION AND CONSERVATION GENETICS OF A HABITAT-SPECIFIC RIVERINE FISH SPECIES, THE EASTERN SAND DARTER (*AMMOCRYPTA PELLUCIDA*).

By

Robert Ginson

A Thesis
Submitted to the Faculty of Graduate Studies through the
Great Lakes Institute for Environmental Research in partial fulfillment of the
requirements for the degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2012

POPULATION AND CONSERVATION GENETICS OF A HABITAT-SPECIFIC RIVERINE FISH SPECIES, THE EASTERN SAND DARTER (*AMMOCRYPTA PELLUCIDA*).

By

Robert Ginson

APPROVED BY:

_____

Dr. Trevor Pitcher
Department of Biological Sciences

_____

Dr. Douglas Haffner
Great Lakes Institute for Environmental Research

_____

Dr. Nicholas Mandrak, Co-Advisor
Fisheries and Oceans Canada

_____

Dr. Daniel Heath, Advisor
Great Lakes Institute for Environmental Research

_____

Dr. Hugh MacIsaac, Chair of Defense
Great Lakes Institute for Environmental Research

June 15, 2012

CO-AUTHORSHIP STATEMENT

I hereby declare that this thesis incorporates material that is result of joint research from co-authored and submitted journal articles undertaken by the supervision of my co-supervisors Dr. Daniel Heath (University of Windsor) and Dr. Nicholas Mandrak (Fisheries and Oceans Canada). The primary contributions, data collections, laboratory work, and interpretation of the data was performed by the author, with additional input on data analysis, interpretation of data, and written discussion by co-authors.

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This thesis includes 1 original paper that has been previously submitted for publication in a peer reviewed journal, as follows:

Chapter 2: Ginson RG, Walter RP, Mandrak NE, Beneteau CL, Heath DD (2012). Range-wide genetic structure and range-edge effects in a habitat-specific freshwater fish species, the eastern sand darter (*Ammocrypta pellucida*). (*Manuscript submitted to Molecular Ecology: June 2012*).

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ABSTRACT

The eastern sand darter (*Ammocrypta pellucida*) is dependent on fine sandy substrates that are naturally fragmented at depositional areas in freshwater lakes and rivers. Loss of suitable habitat is the leading cause of population declines across the entire species distribution. I identified genetic connectivity among drainages, rivers, and populations to determine how eastern sand darter genetic structure is shaped by historic drainage and contemporary river connectivity. Using microsatellite markers, I found that low gene flow among rivers resulted in persistent influences of historic drainage connectivity on current range-wide genetic structure. High within-river genetic connectivity, especially in range-edge rivers, is attributed to extinction/re-colonization events resulting from temporally unstable sand bar habitats, although genetic diversity is preserved through stratified dispersals. Fine-scale and temporal genetic analysis revealed that the Grand River likely represents recent colonization of populations, while the Thames River represents a potentially valuable source for future reintroduction recovery actions.

ACKNOWLEDGEMENTS

I would like to thank Dr. Daniel Heath for his guidance and endless support throughout these past few years, without his supervision this thesis would not have been possible. I would also like to thank Dr. Nicholas Mandrak, who contributed to the planning, execution, and writing of my thesis, his input was always appreciated. I would like to thank my family and friends for their support throughout the years. Thank you to my committee members Dr. Trevor Pitcher and Dr. Douglas Haffner for their time and input.

A special thanks to Jason Barnucz (Fisheries and Oceans Canada), Alan Dextrase (Ontario Ministry of Natural Resources), Nathalie Vachon (Quebec Ministry of Natural Resources), Brant Fisher (Indiana Department of Natural Resources), Douglas Carlson (New York Department of Natural Resources), and Joseph Faber (Ohio Department of Natural Resources) for their insight and assistance with field collections. Finally, I thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and Fisheries and Oceans Canada Species at Risk Program for the main project funding, as well as the University of Windsor and the Ontario Graduate Scholarship for additional funding.

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1.0 GENERAL INTRODUCTION

The sustainability of Earth's biological diversity is of major concern given a changing climate and increasing environmental impacts from anthropogenic sources that continue to be the greatest threat to the maintenance of that diversity (Pereira *et al*. 2010). The global human population has recently surpassed seven billion people, and both direct and indirect consequences of human activities have been associated with the record number of species currently at risk of extinction (Primack 2002). As a result, conservation biology has become an increasingly important area of research, and the conservation of contemporary species biodiversity is not only valuable for their ecological services, but is also vital for the long-term preservation of biological diversity through evolutionary interactions (e.g., speciation) (Primack 2002). Identifying populations at risk of extirpation requires a multi-disciplinary assessment that includes species biology, ecology, demographic life-history, and genetic diversity to develop appropriate conservation and management strategies and approaches (Frankham 2002).The application of genetics to conservation biology is useful for identifying genetically depressed populations, since the loss of genetic diversity can lead to negative genetic effects such as inbreeding depression (Wright *et al.* 2008). Implementing genetic analyses can allow monitoring of negative genetic effects that can pose immediate threats to the evolutionary responsiveness to environmental changes, such as climate change (Frankham 2002).

Recovery actions used for species of conservation interest commonly include reintroduction, supplementation, and introduction (Primack 2002). Reintroduction programs can be useful for re-establishing formerly extirpated populations in restored

habitats, while supplementation programs can be used to augment established, but declining, populations. Introduction programs aim to establish new populations beyond the native range when native habitat becomes uninhabitable. Genetic rescue is a recent addition to the aforementioned actions, and was developed to improve recovery success by supplementing endangered populations using non-threatened, or captive, populations to increase not only population size, but also genetic variability (Hedrick & Frederickson 2010). Genetic rescue, or "genetic restoration", actions are designed to limit the threat of inbreeding depression and the potential loss of locally adapted traits ("outbreeding depression or genetic swamping"), although non-genetic factors such as environment, demography, and species-specific behavior must also be considered (Tallmon *et al*. 2004). Inbreeding depression refers to mating of closely related individuals resulting in a loss of fitness in progeny due to the accumulation of deleterious, recessive alleles, and/or reduced genetic diversity (Bouzat *et al*. 2009). Outbreeding depression acts on future population viability and occurs when genetically differentiated populations hybridize, resulting in a loss of locally adapted genomes (Bouzat *et al*. 2009). For the effective implementation of reintroduction recovery actions, population connectivity must be ensured so that the natural genetic connectivity of populations is retained and so genetic diversity can be maintained for future populations (Friar *et al*. 2000).

Approximately 40% of North American freshwater fish species are considered imperilled and a major reason for the decline of freshwater fish populations is the loss of suitable habitat, primarily as a result of anthropogenic impacts (Jelks *et al*. 2008). The most common anthropogenic influences on freshwater habitats occur as a result of physical barriers, changing stream hydrology (e.g. river straightening), or runoff of

pollutants or sedimentation from either agriculture or urban areas (Jelk *et al.* 2008). Substrate composition within watersheds can be changed by flow alterations from physical impoundments, such as dams or road crossings over streams (Wofford *et al*. 2005). Contamination, from urban or agricultural sources, can alter water and substrate chemistry as well as increase sedimentation within rivers, causing areas of the stream to become uninhabitable by native species (Quinn *et al*. 1997; Meybeck 1998). Finally, non-indigenous species introduced by human activities pose novel threats to native populations through trophic alterations (competition/predation), habitat alterations, disease introductions, and genetic influences (e.g., hybridization) (Dextrase & Mandrak 2006). As suitable habitats become limited and fragmented, genetic exchanges of alleles among populations (gene flow) will be restricted unless dispersal is able to compensate for the increased distances among suitable habitats (Blanchet *et al*. 2010). Limited gene flow among populations will reduce the overall genetic variation within the population and can eventually lead to elevated levels of genetic drift and its associated loss of genetic diversity (Frankham 2002; Neville *et al*. 2006). Although anthropogenic habitat loss can be especially detrimental for some habitat-specific species, compensating life-history characteristics (e.g., enhanced dispersal strategies) can maintain gene flow among populations and preserve genetic diversity within populations (Henle *et al*. 2004). Ultimately, the responses to habitat loss are species-specific, and the degree of among-population genetic differentiation associated with population fragmentation will be largely dependent on life-history traits such as clutch size, longevity, and dispersal strategies (Hoelzel 1999).

The quantitative partitioning of factors that drive population connectivity is important for the conservation and management of species of conservation concern. Determining the spatial scale at which gene flow is limited across a species' range can provide valuable information on many ecological and evolutionary processes (e.g., demographic dynamics, local adaptation potential, patterns of genetic diversity) that may contribute to population stability or extinction (Clobert *et al*. 2001). Additionally, the genetic identification of fine-scale dispersal patterns provides insight into the re-colonization potential of fragmented habitat patches and determines whether gene flow persists among population fragments (Bohonak 1999; Palsbøll *et al*. 2007). Quantifying population connectivity at the large-scale, landscape level also provides valuable information on species range dynamics and helps identify colonization patterns and isolated regions (Costello *et al*. 2003). Temporally unstable changes in geology and climate (e.g., recent Pleistocene glacial retreat) are important factors shaping species range-wide connectivity, and can cause shifts in species distributions (Brown *et al*. 1996). Freshwater species inhabiting formerly glaciated regions (e.g., North American Pleistocene glacial retreat) may still reflect the influence of historical glacial retreats in their genetic structure (Bernatchez & Wilson 1998; Stepien *et al*. 2007). Interpretation of the relative contributions that large-scale, historic processes versus fine-scale, contemporary processes (i.e., anthropogenic barriers) make to current population genetic patterns will facilitate strategies to assess future population viability (Monaghan *et al*. 2002; Stepien *et al*. 2007; Duvernell *et al*. 2008).

Species' range-wide distributions are not only shaped by physical boundaries, where geographic barriers limit population expansion, but range expansions may also be

limited by biotic (e.g., species' interactions) or abiotic (e.g., environmental stress) processes (Brown *et al*. 1996). Species are expected to persist within their environmental tolerance range, and populations located at the centre of a species range have been suggested to inhabit more suitable environments than range edge populations that inhabit marginal habitats (Lesica & Allendorf 1995). Smaller population sizes, higher population isolation, and increased natural selection associated with marginal habitats act together to promote increased genetic drift in range-edge populations and, thus, drive unique patterns of genetic diversity compared to central range populations (Eckert *et al*. 2008). The development of distinct, locally adapted genetic diversity in marginal habitats suggests range-edge populations could be evolutionarily valuable for future species viability resulting from potential range expansion (Lesica & Allendorf 1995). Range expansion beyond the current environmental tolerances requires genetic changes that enable species to adapt to new environmental pressures (Kirkpatrick & Barton 1997). Therefore, range-edge populations that are capable of acclimating to changing, and presumably sub-optimal, environments may play an important role in maintaining and/or increasing species ranges as climate change is expected to promote pole-ward shifts in species ranges due to increasing temperatures (Lesica & Allendorf 1995; Parmesan 2006). Previous range expansions occurring at range-edges have been associated with the development of increased dispersal capabilities (Simmons *et al*. 2004; Bronnenhuber *et al*. 2011).

Distinguishing between natural (e.g., range-expansion) and anthropogenic (e.g., translocation or bait-bucket transfer) newly founded populations is an important process for management and conservation (Beneteau *et al*. 2012). For example, if newly founded

populations represent a non-indigenous species invasion, then the introduced species can have a dramatic and detrimental impact on the native ecosystems (Gozlan *et al*. 2010). On the other hand, gradual range expansion colonisations, often associated with other environmental changes, may have a less deleterious effect on the receiving ecosystem (Beneteau *et al*. 2012). Analyzing the genetic structure of both native and introduced populations of a species will provide insight into the colonization process of newly founded populations (Roman & Darling 2007). Naturally expanding, or introduced, populations are expected to experience population size bottlenecks, as founding populations are made up of a smaller subsample of the source population. Genetic effects associated with small founding populations, or "founder effects", can include a loss of genetic diversity and increased influences of genetic drift. However, the intensity of the genetic bottleneck will vary depending on the size of the source populations as well as the magnitude ("propagule size") and frequency ("propagule pressure") of the introductions (Brown & Stepien 2009). Two colonization mechanisms are suggested for retaining genetic diversity during population introductions: i) multiple introductions, and/or, ii) rapid range-expansions. Newly founded populations can maintain genetic diversity through multiple introductions, which can increase population size, maximize genetic diversity, and minimize genetic drift; additionally, introductions from multiple source populations can result in hybridization between source populations, thus resulting in greater genetic diversity (Kolbe *et al*. 2004; Roman & Darling 2007; Beneteau *et al*. 2012). Similarly, rapid range expansions maintain genetic diversity in newly founded populations by decreasing genetic drift within populations via high levels of gene flow with more range-central populations (Friar *et al*. 2000). To accurately distinguish among

various population introduction pathways, it is essential to utilize a combination of genetic analysis, historic population collection information, and demographic life-history characteristics (Estoup *et al*. 2004).

The eastern sand darter (*Ammocrypta pellucida*) is a small benthic riverine fish species currently listed as Threatened within its entire Canadian distribution by the Committee of Endangered Wildlife in Canada (COSEWIC 2011) and the Species at Risk Act (SARA) largely attributed to expected declines in population sizes and loss of preferred habitat. Eastern sand darter is also listed as a species of special concern in many American states throughout its distribution (Grandmaison *et al*. 2004). The current species range is a patchy network of inhabited, uninhabited, and extirpated rivers that encompasses rivers in the Lake St. Clair, Lake Erie, Lake Champlain, St. Lawrence River, Ohio River, and Wabash River drainages (Grandmaison *et al*. 2004; COSEWIC 2011). Preferred sand bar habitats for eastern sand darter are generally found on the downstream sides of river or stream bends as well as sandy shoals in lakes and form in shallow water (< 0.5m) with water velocities < 0.2 m/s (Daniels 1989). Range-wide population declines are largely associated with the destruction of suitable habitat due to a variety of anthropogenic impacts. Siltation is one of the most severe human impacts on eastern sand darter habitat, and the increased silt likely acts to decrease oxygen availability for burrowing eastern sand darter (COSEWIC 2011).

Eastern sand darter are short-lived with a maximum of 4+ years for individuals in the Thames River (Drake *et al.* 2008) and only 2+ years were determined in Ohio populations, while age-at-maturity occurs at 1+ years (Spretizer 1979; Finch 2009). Spawning has not been observed in the wild, although it is expected to occur in early

June- late July with females able to spawn multiple clutches throughout the summer (Spretizer 1979; Finch 2009). Mean total fecundity for eastern sand darter has been estimated as 343 ova (total number of eggs in Ohio populations) and mean number of mature ova (clutch size) was 71 in Salt Creek (Ohio), 56 in Little Muskingum River (Ohio), and 66 in Thames River (Ontario) (Spreitzer 1979; Faber 2006; Finch 2009). Eastern sand darter lack a swim bladder, which allows the species to exhibit a unique burying behaviour in fine sandy substrates, presumably to reduce the energy expenditure associated with maintaining their position in river flows. Both predator avoidance and improving prey ambush efficiency has been also suggested, but were rejected by Daniels (1989). Dispersal patterns for the species have not been rigorously studied; however, an unpublished tagging study of adult eastern sand darter found no evidence for among-sand bar movements during the summer months (Finch 2009). High genetic differentiation among eastern sand darter populations in the Federal Creek and Hocking River was shown in a previous unpublished study (see Grandmaison *et al*. 2004). However, early life-stage dispersal and/or mixing of separate sand bar populations during the winter months have been suggested, both of which could facilitate population mixing (Simon & Wallus 2006).

1.1 THESIS OBJECTIVE

The goal of this thesis is to characterize the genetic diversity of a habitat-specific fish species at multiple spatial scales to assess the influence of historic drainage processes and contemporary gene flow patterns on genetic structure. I explore the central-marginal species range hypothesis by determining genetic diversity and genetic structure in range-edge populations and comparing them to central range populations. I also identify fine-

scale genetic connectivity and population viability of populations at threat of extirpation, information that will facilitate the implementation of future eastern sand darter recovery strategies.

1.2 CHAPTER 2 OBJECTIVE

Quantifying genetic structure at multiple spatial scales can provide essential information on the relative influence of both historic drainage connectivity and contemporary gene flow patterns on range-wide population connectivity. I test the theoretically accepted, but unverified, genetic characteristics associated with species range-edge populations. Range-edge populations live in marginal habitats and have smaller populations compared to central range populations; therefore, they are expected to exhibit reduced genetic diversity and increased isolation.

The eastern sand darter is a good model species to analyze genetic connectivity at multiple spatial scales across their species range because of their high dependence on naturally fragmented substrates within rivers. Fine spatial-scale fragmentation of substrate, combined with large-scale fragmentation of rivers inhabited by eastern sand darter is expected to promote population fragmentation at multiple spatial scales throughout their distribution.

1.3 CHAPTER 3 OBJECTIVE

In 1994, COSEWIC identified eastern sand darter populations in Canada as Threatened, attributed to declining populations and ongoing anthropogenic loss of suitable sand bar habitats. The status of this species was reassessed in 2000 and 2009 and the Threatened status of populations has been retained, with the addition that Canadian populations be listed as two designatable units (Quebec and Ontario) requiring

independent conservation strategies to be developed (COSEWIC 2011). Since then, Canada's Species at Risk Act has indicated eastern sand darter as Threatened and under Schedule 1 and a proposed recovery strategy has been developed (Fisheries and Oceans Canada 2012). Recovery strategies, such as reintroduction or supplementation, often require information from a variety of biological, ecological, demographic and genetic assessments.

The purpose of this study was to assess current population viability of two southwestern Ontario river eastern sand darter populations and identify within-river gene flow patterns that will provide insight into natural genetic connectivity. Identifying fine-scale population connectivity will provide recovery strategies with an important understanding of the most effective spatial scale for maintaining gene flow and genetic connectivity in reintroduced or supplemented populations. Naturally connected populations in recipient rivers will enable reintroduced populations to maintain effective population sizes and genetic diversity for future population viability.

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2.0 RANGE-WIDE GENETIC STRUCTURE AND RANGE-EDGE EFFECTS IN A HABITAT SPECIFIC FRESHWATER FISH SPECIES, THE EASTERN SAND DARTER (*AMMOCRYPTA PELLUCIDA*)[1]

2.1 INTRODUCTION

Ecological communities are shaped in varying degrees by abiotic (e.g., physical/chemical) and biotic (e.g., predation/competition) factors, as well as spatial landscape effects (Jackson *et al*. 2001). Landscape-level dispersal patterns provide networks of population connectivity that are important for not only regional abundance and distribution of species, but also the future persistence of populations (Turner *et al*. 1989). Quantifying population connectivity at multiple spatial scales allows interpretation of the relative contribution that large-scale historic processes (e.g., climate, geography) and contemporary fine-scale processes (e.g., barriers) make to population ecology processes and patterns (Wiens 1997; Monaghan *et al*. 2002). Molecular genetic methods can successfully characterize many aspects of freshwater ecosystem processes and connectivity including landscape effects on genetic sub-structure (Cook *et al*. 2007; Caldera & Bolnick 2008), historical influences on contemporary population structure (Poissant *et al*. 2005; Stepien *et al*. 2007; Boizard *et al*. 2009), colonization patterns and alternative dispersal pathways (Mäkinen *et al*. 2006), and species introductions (Dlugosch & Parker 2008; Beneteau et *al*. 2012). Genetic identification of fine-scale dispersal provides insight into gene flow patterns among fragmented populations as well as the re-colonization potential of fragmented habitat patches (Bohonak 1999, Palsbøll *et*

[1] Ginson RG, Walter RP, Mandrak NE, Beneteau CL, Heath DD (2012). Range-wide genetic structure and range-edge effects in a habitat-specific freshwater fish species, the eastern sand darter (*Ammocrypta pellucida*). (*Manuscript submitted to Molecular Ecology: June 2012*).

al. 2007). Measuring gene flow is especially important as it interacts with other evolutionary forces such as genetic drift, mutation, and natural selection to mediate evolutionary change (Bohonak 1999). Quantifying population connectivity at the landscape level provides valuable information on species range dynamics and aids in the identification of isolated populations requiring special conservation attention (Manel et al. 2003; Cook *et al*. 2007; Storfer *et al*. 2007).

Population connectivity largely depends on species-specific dispersal capabilities (Watanabe *et al.* 2010) and dispersal barriers, which can limit among-population movements and thus disrupt genetic processes such as migration-drift equilibrium (McGlashan & Hughes 2001; Poissant *et al.* 2005; Johansson *et al*. 2008). Freshwater ecosystems often experience high levels of fragmentation resulting from dispersal barriers, because such systems generally rely on linear corridors of stream connectivity (Ward *et al*. 1994). The diversity of freshwater connectivity pathways, ranging from small streams to large flowing rivers to lakes, provides a variety of possible dispersal barriers for freshwater organisms (Caldera & Bolnick 2008). Additional barriers mediating dispersal in freshwater ecosystems include extrinsic factors (such as anthropogenic disturbances, water flow rates, and stream gradients; Matthews & Robinson 1998; Hänfling & Weetman 2006; Caldara & Bolnick 2008) and intrinsic factors (such as loss of fitness in migrants and local adaptation promoting reproductive isolation: Beheregaray & Sunnucks 2001; Nosil *et al*. 2005). Species dependent on specialized habitats may be at higher risk for negative effects resulting from habitat disruption as this can generate additional gene flow barriers when dispersal opportunities are already limited (Templeton *et al.* 1990; Johansson *et al*. 2008). Some habitat-specific

darter species experience increased extinction/re-colonization rates due to the loss of specialized habitat, consequently disrupting the development of within-river genetic structure (Turner & Trexler 1998).

While dispersal patterns at a local scale have been well studied in freshwater fish (e.g., Hänfling & Weetman 2006; Beneateau *et al.* 2009; Haponski *et al.* 2009), range-wide population dynamics theory has been relatively poorly tested. In theory, range-edge populations are predicted to experience increased genetic drift resulting from small population sizes and elevated isolation due to low suitable habitat availability and, therefore, increased genetic differentiation among populations (Lesica & Allendorf 1995). Although increased genetic drift can eventually promote a loss of genetic diversity within populations, if genetic diversity persists among populations it could be evolutionarily important for adaptation to environmental change (Hutchison 2003). Additionally, unique genetic variation in range-edge populations may result from local adaptation to marginal habitats (Lesica & Allendorf 1995). As climate change is expected to promote pole-ward shifts in species ranges due to increasing temperatures, range-edge populations capable of acclimating to the changing environment may play a role in maintaining or increasing species ranges (Chu *et al*. 2005; Parmesan 2006). A review of peripheral population studies by Eckert *et al*. (2008) found that various plant and animal species displayed lower genetic diversity and increased levels of differentiation in range-edge populations compared to central populations, which corresponds with the central-marginal hypothesis. The central-marginal hypothesis predicts that populations closer to the centre of the species range will have better habitats compared to range-edge populations that experience fragmentation from marginal environments (Eckert *et al*.

2008). The abundant centre model states that species have their highest population sizes in the centre of their range and is the underlying principle for range-edge genetic diversity loss; however, few empirical studies have unambiguously demonstrated this model (Sagarin & Gaines 2002).

The eastern sand darter (*Ammocrypta pellucida*) is a small benthic riverine fish species that is listed as Threatened federally in Canada and in many regions of its American distribution (Grandmaison *et al*. 2004; COSEWIC 2011). The eastern sand darter exhibits a unique burying behaviour in sandy substrates, and, while not entirely understood, has been suggested by Daniels (1989) to serve to reduce energy expenditure associated with maintaining position in flowing rivers. A tagging study of adult eastern sand darter found no evidence of among-sand bar movements during the summer months (Finch 2009) and, thus, the patchy distribution of sand bar habitats within rivers and streams is expected to promote fine-scale population fragmentation. However, early life-stage dispersal and/or mixing of separate sand bar populations during the winter months have been suggested, but not tested, and both possibilities would facilitate population mixing (Simon & Wallus 2006). At a larger scale, the species range is a patchy network of inhabited and uninhabited rivers and loss of suitable habitat has been attributed to anthropogenic pressures in most river systems (Grandmaison *et al.* 2004; COSEWIC 2011). The current species range encompasses rivers in the following drainages; 1) Lake St. Clair, 2) Lake Erie, 3) Lake Champlain, 4) St. Lawrence River, 5) Ohio River, and 6) Wabash River (Fig. 2.1: Grandmaison *et al*. 2004; COSEWIC 2011).

Here, we examine population fragmentation, range-wide connectivity, and genetic structure for a habitat-specialist freshwater fish species. Using microsatellite genotype

data at 10 loci in fish from 39 sites sampled across the species range, we quantify genetic connectivity of eastern sand darter populations at multiple spatial scales. Our specific objectives are: (1) characterize contemporary population connectivity by analyzing genetic structure; (2) determine the relative influence of historic (post-glaciation) colonization patterns versus current connectivity processes on the drainage genetic structure; and, (3) test the central-marginal hypothesis predictions for differences in genetic diversity and isolation among central range and range-edge populations. Overall, we expect high genetic structure for this species, even at small spatial scales because of their dependence on fragmented sandy substrate habitats. We expect to see genetic isolation effects in range-edge populations of the Great Lakes, compared to the centrally located Ohio River drainage, as the range-edge population experience an increased threat of population extirpation associated with population losses (Grandmaison *et al*. 2004; COSEWIC 2011). Consequently, the combination of habitat fragmentation within rivers, disjunction of occupied rivers throughout the species range, and declining population sizes in most inhabited rivers reinforces the conservation and evolutionary importance of characterizing connectivity among eastern sand darter populations.

2.2 MATERIALS AND METHODS

Sampling protocol: Sampling efforts were directed at rivers recently reported to harbour eastern sand darter populations, according to Canadian and American government status reports (Fisheries and Oceans Canada 2011; Grandmaison *et al*. 2004), and on sand bars at depositional bends within those rivers. Hierarchical sampling definitions used in this study are: sample sites (e.g., HR1) are located within rivers (e.g., Hocking River), and located within drainages (e.g., Ohio River drainage). Sampling occurred in four drainages

across the species range (Fig. 2.1): i) Ohio River drainage (Little Muskingum River, Hocking River, Salt Creek, Red River, Licking River); ii) Wabash River drainage (Eel River, East Fork White River, Deer Creek, Big Creek); iii) Great Lakes drainage (Maumee River, Grand River, Thames River, Sydenham River),; and, iv) St. Lawrence River (Richelieu River, Rivère au Saumon, Champlain Canal). Fish were caught with a bag seine net (dimensions: wings 15m x 3m with 0.64cm mesh and 1.5 x 1.5 x 1.5m bag with 0.32cm mesh) or by using a Missouri trawl specialized for benthic fish collection (J. Baruncz, pers. comm., Fisheries and Oceans Canada, Burlington, ON). Upon collection, a small pelvic fin clip was taken from each fish and preserved in 95% ethanol for subsequent DNA analysis. After a short recovery period in freshwater recovery tanks, fish were then returned to their original habitats.

DNA extraction and genotyping: The study used ten microsatellite primers, five of which were developed for other species (Esc132b, EosC6, EosC112, EosD107, EosD11) and an additional five primers (Esd3, Esd13, Esd17, Esd18, Esd25) were developed specifically for eastern sand darter. To develop the primer sets, extracted eastern sand darter DNA was enriched for microsatellite repeat sequences according to a protocol adapted from Fischer and Bachman (1998). Genomic DNA was digested with RsaI and the blunt ends were then ligated to MluI adapter-primer complexes. Segments were then hybridized with biotinylated oligo ($GACA_4$) probes and captured with streptavidin-coated beads (Roche, Indianapolis, USA). The resulting enriched DNA fragments were cloned into TOPO vectors and then transformed into One Shot competent *Escherichia coli* cells (Invitrogen, Burlington, Canada). Inserts from the clones were amplified using M13 universal forward and reverse primers and sequenced at the Genome Quebec Innovation Centre (McGill

University, Montreal, Canada). Microsatellite primer pairs were designed and optimized for polymorphism and ease of amplification using Polymerase Chain Reactions (PCR). PCR amplification of all ten microsatellite loci used in this study was performed in 12.75µL reactions containing approximately 50-100ng template DNA, 0.25µL of 0.5µM dye-labelled forward primer, 0.25µL of 0.5µM reverse primer, 200µM of each dNTP, various concentrations of $MgCl_2$ (see Appendix 2.1), and 0.25U Taq DNA polymerase (Applied Biosystems, Foster City, USA) in a 1X PCR buffer. The thermal cycler profile was an initial denaturing period at 94^{o}C for 120 seconds followed by 35 cycles of 94^{o}C for 30 seconds, various annealing temperatures for each primer (Appendix 2.1) for 45s, 30s at 72^{o}C, and 90s at 72^{o}C at the final extension period. Dye-labelled PCR products were visualized on a LiCor 4300 DNA analyzer (Li-COR Biosciences, Inc.) polyacrylamide gel with 3 out of 67 lanes containing manufacturers' size standard (50-350bp). To determine individual genotypes, Li-COR gels were scored for allele size using GENE IMAGIR 4.05 software (Scanalytics Inc.).

Genetic marker validation: Genotype data for each site were tested for the presence of null alleles, allele scoring error, and large allele drop-out using MICROCHECKER v2.2.3 (Van Oosterhout *et al*. 2004). All pairs of microsatellite loci were analyzed for linkage disequilibrium using ARLEQUIN v3.01 (Excoffier *et al*. 2005). Departures from Hardy-Weinberg equilibrium (HWE) were assessed for all possible locus-by-site combinations using the Markov chain Monte Carlo (MCMC) method (100000 dememorisation steps; 1000000 Markov Chain steps) in ARLEQUIN. Inbreeding coefficients (F_{IS}), averaged across all 10 loci, according to Weir and Cockerham (1984) were also calculated in ARELQUIN. HWE departure significance, and other pairwise

comparisons below, were adjusted for multiple simultaneous tests using sequential Bonferroni correction (Rice 1989).

Genetic structure:

Genetic differentiation: Genetic differentiation was quantified by calculating pairwise F_{ST} values (Weir & Cockerham 1984) among all sites within each sampled river using ARLEQUIN. Genetic distance among sites was estimated by genetic chord distances (D_C; Cavalli-Sforza & Edwards 1967), which do not assume any mutation model, using POPULATIONS v1.2.28 (Langella 2002). Finally, all pairwise within-river sites were tested for allele frequency distribution differences using exact tests with 10 000 permutations (Raymond & Rousset 1995) in TFPGA v1.3 (Miller 1997). To quantify genetic differentiation among rivers for all four drainages, sites within each river were combined and mean pairwise F_{ST} estimates were calculated among rivers using ARLEQUIN. Genetic differentiation was also compared among drainages by calculating global F_{ST} values for each drainage, with significance determined by jackknifing across all loci at the 95% confidence interval in FSTAT (Goudet 2001).

Migration-drift equilibrium: Rivers containing at least three sampling sites separated by at least five kilometres were tested for adherence to an isolation-by-distance (IBD) model of migration-drift equilibrium, as proposed by Hutchison and Templeton (1999). IBD was determined using the association between linearized genetic differentiation (F_{ST}/1-F_{ST}) and hydrological distances (km) among sites, with a Mantel test for significance (9 999 permutations) in GENALEX 6.0 (Peakall & Smouse 2006). Drainage-level IBD, with a Mantel test for significance as above, was also determined using linearized genetic differentiation [F_{ST}/(1-F_{ST})] among all sites and the shortest hydrological distances

between sites using GIS. However, eastern sand darter prefer shallow, sandy habitats so hydrological distances were determined using two methods: littoral restriction (assumes individuals avoid open water and calculates shoreline distances through lakes) and, open-water dispersal (uses the shortest water distances among rivers including dispersal through open water).

Range-wide genetic connectivity: An analysis of molecular variance (AMOVA) was used to hierarchically partition genetic variation within each drainage into three levels: among rivers; among sites within rivers; and, within sites using ARLEQUIN. We also identified the number of population genetic clusters based on underlying genetic similarity, without assuming geographical association, using the Bayesian-based clustering program STRUCTURE (v.2.3.1 (Pritchard *et al.* 2000). STRUCTURE assigns individuals into inferred clusters based on microsatellite genotypic data and was run with a 30 000 burn-in period, 100 000 Markov chain Monte Carlo (MCMC) generations, with 3 iterations (allele frequencies correlated and potential admixture allowed). The allowed number of genetic populations ranged from K = 1 (suggesting total population panmixia) to the total number of rivers plus one (K = 17) to ensure the true number of genetic clusters was included. Second-order rate of change (ΔK) of the LnP(D) function was used to select the most likely value of K (Evanno *et al*. 2005). To corroborate the genetic clusters identified in STRUCTURE, we performed a principal coordinate analysis (PCoA) for all sites using a pairwise matrix of F_{ST} values in GENALEX. PCoA shows the genetic relationships among sites without the genetic equilibrium assumptions of STRUCTURE. To identify breaks in gene flow patterns among geographically close sites, BARRIER v2.2 (Manni *et al*. 2004) was implemented using the landscape genetic approach of

Monmonier`s maximum difference algorithm across the range. In BARRIER, pairwise estimates of F_{ST} were mapped onto a matrix of their geographic coordinates (latitude and longitude), and a Monmonier maximum-difference algorithm identified which of the borders between neighbouring populations exhibited the highest level of genetic divergence.

Genetic structure hypotheses:

Contemporary versus historic influences: As historic colonization patterns can confound contemporary connectivity patterns, population genetic structure should be analyzed at multiple spatial scales for confident interpretation of population connectivity (Duvernell *et al*. 2008).

To identify contemporary among-river dispersal, we performed an individual-based assignment method to assign all sampled individuals to their source rivers using the partial Bayesian method of Rannala and Mountain (1997) in GENECLASS 2.0 (Piry *et al*. 2004). Individuals with assignment likelihood values less than 0.10 were excluded from the analysis as likely having come from unsampled rivers. We identified the most likely source river for each fish using the rank-based assignment method of GENECLASS to determine the proportion of individuals assigning to rivers and drainages, other than their river of capture. Migrants were identified using the criterion that the highest assignment probability was greater than nine-times the assignment probability for any other river to minimize the potential for false among-river dispersal identification.

To determine the influence of historic drainage connectivity on contemporary genetic structure, we tested the relative partitioning of genetic variation identified by

historic versus contemporary groups of sites using an Analysis of Molecular Variance (AMOVA) implemented in ARLEQUIN. The contemporary hypothesis grouped sites based on current drainage connectivity; therefore, sites were grouped into: i) Great Lakes; ii) Ohio River and Wabash River; and, iii) St. Lawrence River. The historic connectivity hypothesis grouped the sites based on genetic clusters identified by STRUCTURE, PCoA, and BARRIER and sites were grouped into: i) Great Lakes and Wabash River; ii) Ohio River; and, iii) St. Lawrence River. The proportion, and significance, of the genetic variance partitioned into the groups described by each hypothesis was assessed hierarchically using AMOVA.

Range-edge effects: To determine if populations experience range-edge influences on genetic structure patterns, we compared within-river genetic differentiation for four rivers containing multiple sites (> 3 sites) and classified them as northern boundary (TH and GR) or central range (HR and MA). We excluded the St. Lawrence River sites from within-river analysis as sampling success was low. As within-river sampling success was also low in the southern range edge, we were not able to analyze genetic differentiation for this boundary. Within-river migration-drift equilibrium at the northern boundary and central range sites was determined using IBD for the same northern range-edge and central rivers. We compared dispersal patterns between northern range-edge rivers (TH & GR) and central range rivers (HR & MA). To do so, we used the partial-Bayesian individual assignment method in GENECLASS to exclude fish that failed genetic assignment to any site ($P < 0.10$). We then implemented the rank-based method in GENECLASS to identify the most likely source site for each successfully assigning

individual. We used a sensitivity analysis for the rank-based approach to identify the appropriate threshold ratio of highest likelihood to second highest.

To test the central-marginal range hypothesis that range-edge populations contain lower genetic variation than centrally located populations, genetic diversity estimates were compared among all sample sites. Genetic diversity was estimated as expected heterozygosity (H_E) and corrected allelic richness (A_R) using FSTAT. To test for significance, mean allelic richness and expected heterozygosity for all range-central and range-edge pairwise site comparisons were performed using the Mann-Whitney U test for independent, non-parametric samples in SPSSv10.0.7 (SPSS INC.). To identify whether populations closer to the range-edge exhibited lower genetic diversity, we compared the allelic richness estimates across all sampled latitudes and longitudes. We also tested for genetic evidence of recent changes in population size to explore the hypothesis that range-edge populations experience frequent and ongoing population bottlenecks or founder effects (due to range expansion), using the program BOTTLENECK (Piry *et al*. 1999). In BOTTLENECK, we used a Bayesian approach to the stepwise mutation model (SMM) and the two-phase mutation (TPM) model, suggested to be most appropriate method for microsatellite data, to determine whether any of the sample sites contained excess heterozygotes, reflective of a recent population size contraction. To test for the statistical significance of identified bottlenecks, BOTTLENECK uses a two-tailed Wilcoxon signed-rank test compared to the expected normal distribution of heterozygosity under mutation-drift equilibrium. The allele frequency distribution of each population is then established to determine if populations experience a “mode-shift” from the normal L-shaped distribution, which would represent a population bottleneck. We

also analyzed each site for historic population bottlenecks by calculating the mean ratio (across loci) of the number of alleles to the range in allele size using the "M-ratio" test in M_P_Val.exe (Garza & Williamson 2001). M-values are negatively correlated with the duration and severity of the bottleneck.

2.3 RESULTS

Sampling and marker assessment: A total of 1051 eastern sand darter were collected from 16 rivers across the entire species range over an 18-month period from June 2010 to November 2011 (Fig. 2.1). All microsatellite loci used were variable, ranging from 8 to 70 alleles (Appendix 2.1). Significant departures from HWE were found in 8 out of 390 possible locus-by-site combinations following Bonferroni correction ($P < 0.001$) (Table 2.1). Five populations (HRc1, HRc2, HRm3, HRm1, LK) were monomoporhic at Esd3, while CC was monomorphic at EosC6. Seven of the locus-by-site deviations from HWE were attributed to null alleles by MICROCHECKER, with no single locus having more than two sites deviating from HWE. As there is little evidence for an association between a locus containing potential null alleles and sites deviating significantly from HWE, we suggest that null alleles are not influencing our results. Significant ($P < 0.001$) linkage disequilibrium was determined for five out of 390 possible locus-by-locus combinations over all the sites, with no two loci identified as significantly linked for more than one site; therefore, we conclude that the marker loci used in this study are unlinked.

Genetic structure:

Genetic differentiation: Within-river pairwise F_{ST} values among sites ranged from -0.003 to 0.085 in the Ohio River drainage, -0.007 to 0.024 in the Great Lakes drainage, and was 0.005 in the Richelieu River (St. Lawrence drainage) (Table 2.2). Only two rivers

contained significant pairwise F_{ST} values among sites (2/10 in MA and 3/10 in HR) following Bonferroni correction (Table 2.2). Significant pairwise exact tests were highest in Maumee River with 80% (8/10) of the tests significant ($P < 0.05$); however, this river also exhibited lowest range of chord distances (0.16-0.21; Table 2.2). The Thames and Grand Rivers had 46.7% (7/15) and 33.3% (5/15) significant pairwise exact tests, respectively, with D_C values ranging from 0.24 to 0.29. Similar proportions of significant among-site exact tests (43.8%, 7/16 tests) and D_C values (0.20 to 0.29) were found in the Ohio River drainage (Table 2.2). Pairwise F_{ST} values among rivers within each drainage also revealed significant genetic differentiation as values ranged from 0.009 to 0.085 in the Wabash River, 0.032 to 0.081 in the Ohio River, 0.021 to 0.090 in the Great Lakes, and 0.060 to 0.18 in the St. Lawrence River drainages (Appendix 2.2). Only three among-river combinations were not significant following Bonferroni correction, two of which were located in the Wabash River drainage (BC-DC, BC-EF). The only Great Lakes combination without significant genetic differentiation occurred between the Thames and Sydenham Rivers (Appendix 2.2). All pairwise exact tests of differentiation resulted in significant values and similar D_C value ranges were found in all three regions (0.25 to 0.37 WR; 0.31 to 0.42 OR; 0.26 to 0.41 GL; 0.31 to 0.47 SL) (Appendix 2.2). Global F_{ST} values for all drainages revealed that the St. Lawrence region had the highest overall genetic differentiation ($F_{ST} = 0.11 \pm 0.022$) compared to the other drainages (GL $F_{ST} = 0.049 \pm 0.011$; OR $F_{ST} = 0.054 \pm 0.011$; WR $F_{ST} = 0.044 \pm 0.014$, even after geographic distances were corrected to 1 000km (SL $F_{ST} = 0.44$; GL $F_{ST} = 0.099$; OR $F_{ST} = 0.090$; WR $F_{ST} = 0.069$).

Migration-drift equilibrium: Due to limited numbers of within-river sample sites, IBD was only assessed in the three rivers (MA, GR, TH) in the Great Lakes drainage and two of the rivers (SC, HR) in the Ohio River drainage. Significant within-river IBD (P = 0.039) was found for the Maumee River as indicated by the low gene flow between high hydrologic distances (R^2 = 0.61). No significant IBD was determined for Hocking River (HR), although the analysis was strongly influenced by HRc1 (upper site in HR creek) and the correlation between gene flow and hydrologic distance was much lower with the site included (R^2 = 0.053, P = 0.27) than without it (R^2 = 0.50, P = 0.082). No significant IBD correlation was determined for Salt Creek (SC: P = 0.33); however, this river only has three sample sites. Low F_{ST} values among all sites indicated high gene flow among all sites in the Thames and Grand Rivers, even when sites were separated by 90 km, resulted in a lack of IBD correlation for both rivers (R^2 = 0.035, P = 0.21 and R^2 = 0.021, P = 0.21, respectively). As described in Chapter 3, the Thames River revealed significant IBD with an increased number of sampling sites. Mantel tests of IBD among rivers, within drainages, showed that both the Ohio River (R^2 = 0.18, P = 0.004) and Great Lakes (R^2 = 0.80, P = 0.0001, straight-line and R^2 = 0.79, P = 0.0001, littoral distances) drainages had significant IBD, although gene flow was more strongly influenced by the hydrologic distances among rivers in the Great Lakes drainage as indicated by the higher Mantel test slope (Fig. 2.2). Neither the Wabash River (R^2 =0.79, P = 0.125) nor St. Lawrence River (R^2 = 0.52, P = 0.084) drainages adhered to an IBD pattern, although both contained only four sampled sites (Fig. 2.2).

Range-wide genetic connectivity: AMOVA for each drainage revealed low partitioning of the genetic variation among sites within rivers: Ohio River drainage (0.42%, P = 0.002),

Great Lakes drainage (0.31%, P = 0.008), St. Lawrence River drainage (0.46%, P = 0.132). However, substantial genetic variation was attributed among rivers in all drainages: Ohio River drainage (6.50%, P < 0.0001), Great Lakes drainage (6.29%, P < 0.0001), St. Lawrence River drainage (10.52%, P < 0.0001). The highest proportion of genetic variation in all analyses occurred within sites: Ohio River drainage (93.085, P < 0.0001), Great Lakes drainage (93.39%, P < 0.0001), St. Lawrence River drainage (89.02%, P = 0.116). The Wabash River drainage was excluded from the AMOVA analysis because of limited within-river sampling sites. STRUCTURE revealed two possible grouping patterns with approximately equal probability (based on Delta-K criteria; Appendix 2.3). STRUCTURE showed that sites from the Wabash River drainage were grouped into a single genetic cluster with the Great Lakes and St. Lawrence River drainages at K = 2 (Fig. 2.3a) while the Ohio River drainage sites grouped separately. Delta K values identified a second genetic clustering of sites at K = 7, where STRUCTURE revealed genetic clusters that strongly reflected the sampled rivers (Fig. 2.3a). At K =7, all within-river sites were clustered together while only a few rivers in each drainage were grouped as a single cluster (Fig. 2.3a). Principal coordinate analysis (PCoA) revealed a similar separation between sites located in the Ohio River drainage versus the rest of the range-wide sites along the first axis (Fig. 2.3b). The PCoA also showed a clear separation of the St. Lawrence River drainage from the rest of the sampling sites (Fig. 2.3b). PCoA corroborated the results from STRUCTURE, as two Wabash River sites (ER/EF) clustered closely with the Great Lakes drainage, while the other sites (DC/BC) clustered closer to the Ohio River drainage. The first two axes of the PCoA accounted for 62.7% of the total genetic variance of our sites (axis1, PC1 = 44.3%,

axis2, PC2 = 18.4%). BARRIER identified three major genetic breaks: the first separated the Ohio River drainage from the rest of the range; and, the second genetic barrier isolated the Champlain Canal site from all other sites (Fig. 2.1). The third genetic barrier isolated the St. Lawrence River drainage from the Great Lakes drainage (Fig. 2.1).

Genetic structure hypotheses:

Historic versus contemporary connectivity: GENECLASS successfully assigned 807 individuals with 2.5 % (20/807) of the individuals identified as among-river migrants. However, nine of the identified among-river migrants were determined to be biologically unlikely because they occurred between drainages that have little or no potential for natural dispersal. Otherwise, among-river migrants ranged from a low 0.5 % in the Grand River to a high of 5 % in Deer Creek and Big Creek (Table 2.3).

AMOVA results for both historic and contemporary hypotheses yielded highly significant among-group variance components, however, a greater proportion of the among-groups genetic variance was explained when the groups reflected the historic connection between the Wabash River and Great Lakes drainages (8.15%, $P < 0.0001$), as opposed to the contemporary connectivity (5.09%, $P < 0.0001$). In both AMOVA analyses, a substantial component of the genetic variance was attributed to within-river variations (historic = 86.6 % and contemporary = 87.8 %, $P < 0.0001$ for both).

Range-edge effects: Among-site pairwise F_{ST} values revealed that 25.0% (5/20) of the site comparisons within the central range rivers were significant, whereas, no (0/30) among-site significant pairwise differentiation was determined in either northern range-edge river (TH or GR). Low genetic differentiation in the northern range-edge rivers resulted in disrupted IBD, whereas, one central range river (HR) exhibited disrupted IBD while

the other tested river (MA) showed significant (P = 0.038) correlations between genetic differentiation and increasing hydrological distances. GENECLASS identified 33 migrants out of 358 individuals successfully assigning to any of our sampling sites, according to the 4:1 threshold (Table 2.4). As our choice of 4:1 is arbitrary, we performed a sensitivity analysis to assess the effect of our likelihood ratio choice. The sensitivity test included likelihood ratios ranging from 2:1 to 9:1 and, although the number of successfully assigned fish decreased, the pattern of migrants did not change appreciably until the ratio was greater than 4:1 (Appendix 2.4). The river with the highest percentage of among-site migrants was the Hocking River with 13.2% (10/76) and the lowest percentage of among-site migrants was found in the Grand River 5.95% (5/84) (Table 2.4).

Global Hardy-Weinberg exact tests and inbreeding coefficients revealed that only three sites experienced significant (P < 0.005) heterozygote deficiencies (Thu1, Thu2, Rd), all of which occurred in range-edge sites (Table 2.1). When A_R was plotted against latitude and longitude, the Champlain Canal, Richelieu River, and Rivière au Saumon showed anomalously low genetic diversity values relative to the other sites (Fig. 2.4). However, Mann-Whitney U tests for significant differences in A_R and H_E found that only the Champlain Canal exhibited significantly lower genetic diversity based on pairwise river comparisons. There was little evidence for recent bottlenecks based on heterozygosity estimates within any of the range-wide sites according to both the SMM (P = 0.59 to 0.99 OR; 0.72 to 0.99 WR; 0.59 to 0.99 GL; 0.71 to 0.99 SL) and the less stringent TPM (P = 0.16 to 0.99 OR; 0.38 to 0.78 WR; 0.012 to 0.95 GL; 0.33 to 0.92 SL) models in BOTTLENECK. MA1 was the only site with a significant heterozygosity

excess detected (P = 0.012); however, no shift in the normal L-shaped allele frequency distribution was identified by BOTTLENECK. M-values in all sites were similar and values ranged from 0.60-0.81 (Table 2.1).

2.4 DISCUSSION

Our study demonstrated extensive genetic connectivity among eastern sand darter populations within all rivers, regardless of anthropogenic barriers (e.g., low Grand River genetic differentiation despite separation of sites by a dam). The nature of freshwater landscapes often promotes low within- but high among-river genetic structure for freshwater fish populations (Mäkinen 2006; Cook *et al*. 2007; Shikano *et al*. 2010). However, high within-river genetic structure has been observed in habitat-specific species (e.g., Hänfling & Weetman 2006; Beneteau *et al*. 2009), mainly as a result of anthropogenic barriers. Low within-river genetic structure was not expected for our species as populations are fragmented due to both natural habitat fragmentation and anthropogenic loss of suitable habitat. Genetic drift is expected to be higher in small fragmented populations of species with short generation times and high dependence on specific substrates (Henle *et al*. 2004), although small-bodied fish species can reduce the genetic impacts of fragmentation via species-specific dispersal abilities or life-history characteristics (Blanchet *et al.* 2010; Slack *et al.* 2010). Furthermore, larger-bodied fish species can have smaller population sizes and unstable population dynamics compared to lower trophic-level species, such as the eastern sand darter (Henle *et al.* 2004; Blanchet *et al*. 2010). As eastern sand darter population sizes are in decline throughout most of their range, we suggest that the lack of genetic structure within most range-wide rivers likely

reflects species-specific dispersal patterns and/or life history characteristics that act to maintain genetic connectivity.

Our study found high genetic structure among rivers, in all sampled drainages, as was expected for small-bodied freshwater fishes, since among-river dispersal can be restricted by large flowing rivers and unsuitable lake habitats (Cook *et al*. 2007; Zambudio *et al*. 2009). Both greenside darter (*Etheostoma blennioides*; Beneteau *et al*. 2009) and rainbow darter (*Etheostoma caeruleum*; Haponski *et al*. 2009) exhibited substantial genetic divergence among rivers within the same Great Lakes drainage as our study. It is expected that a variety of biotic (e.g., predation, competition) and abiotic (e.g., stream morphology, water chemistry) freshwater stream characteristics restrict the ability of fish to disperse freely throughout drainages, as is likely the case for darter species (Jackson *et al*. 2001). Although not tested here, we expect that separation of rivers by largely unsuitable lake habitats, high river flows in the mainstem Ohio River and Wabash River drainages, and enormous hydrological distances separating inhabited rivers also restricts eastern sand darter movement among rivers. Only three exceptions to significant among-river genetic divergence were found; the first occurred between the Thames and Sydenham Rivers and the other two were found in the Wabash River drainage. Low genetic differentiation between the long-established Thames (since 1923) and Sydenham (since 1927) Rivers can be explained by: i) ongoing dispersal through Lake St. Clair; or, ii) headwater connections (natural floods or anthropogenic fish movement). The low number of among-river migrants, combined with the overall high genetic differentiation among rivers, suggests that dispersal through Lake St. Clair is unlikely, and that the genetic similarity between these two rivers may reflect a headwater connection or bait-

bucket transfer, as had been suggested for greenside darter (Beneteau *et al*. 2009). Genetic connectivity among rivers in the Wabash River drainage compared to the other drainages likely results from fewer anthropogenic barriers (e.g., dams in the Ohio River), lower flow rates compared to the Ohio River, and/or smaller hydrological distances separating rivers compared to the Great Lakes and St. Lawrence River drainages.

Despite significant IBD in the Maumee River, hydrological distances among sites were not a reliable predictor of genetic connectivity within most rivers. Absence of IBD was generally attributable to low genetic differentiation among sample sites in most rivers, and this pattern was especially prevalent in the Thames and Grand Rivers (although no IBD in the Thames River in Chapter 3). Over time, within-river IBD is expected to form unless dispersal distances are larger than the spatial extent of the study area, or if sufficient long-distance dispersal events occur to swamp genetic drift effects (McGlashan & Hughes 2001). Our migrant analysis revealed that persistent dispersal occurred within all analyzed rivers, although some had fewer than others (Table 2.4) and, in all rivers; there was evidence of rare, long-distance dispersal events. A combination of long and short dispersal strategies (or "stratified dispersal") will act to buffer against genetic drift and loss of genetic diversity within rivers, consequently restricting within-river genetic structure (Bronnenhuber *et al*. 2011). Within-river movements may be also influenced by temporally unstable habitats, wherein populations are forced to disperse throughout the river when local preferred habitat is lost, a likely scenario for sand deposition-based habitat. Habitat availability and annual discharge have previously been shown to have strong influences on life history characteristics (e.g., growth) in juvenile eastern sand darter (Drake *et al*. 2008). Eastern sand darter may also experience a

pelagic larval stage where downstream drift could facilitate gene flow within rivers (Simon & Wallus 2006). Finally, disrupted within-river IBD may be a result of multiple population bottlenecks, preventing equilibrium between migration and drift as has been suggested for other darter species (Turner & Trexler 1998; Johnson *et al*. 2006). Although BOTTLENECK results did not provide strong evidence for recent population declines, M-values (Table 2.1) were often lower than the 0.68 threshold purposed to represent significant population bottlenecks (Garza & Williamson 2001). Therefore, the lack of genetic structure in river populations of eastern sand darter reflects a combination of stratified dispersal and intermittent local population bottlenecks, both of which can likely be explained by unstable preferred habitat driving increased within-river dispersal and founder effects.

The Grand River eastern sand darter showed little dispersal compared to the Thames and Maumee Rivers and, thus, the very low genetic divergence among sites in the Grand River is unexpected. Low genetic differentiation, coupled with high genetic diversity, may be explained by a recent range expansion of this species into the Grand River, as has been suggested for greenside darter in the Grand River (Beneteau *et al.* 2012). Beneteau *et al*. (2012) suggested that greenside darter populations were introduced just prior to 1990; interestingly, eastern sand darter had not been identified in the Grand River until 1987 (COSEWIC 2011), raising the possibility that the two species may have experienced similar introductions. Species introductions are expected to result in reduced genetic diversity through founder effects resulting from small propagule size (Dlugosch & Parker 2008), although it has been recognized that multiple introductions can preserve genetic diversity (Beneteau *et al*. 2012) or facilitate rapid population expansion (Roman

& Darling 2007). As in the case with the greenside darter, naturally occurring range expansion of eastern sand darter appears unlikely due to large open-lake water distances and in-stream barriers separating the Grand River from the nearest source for colonization (COSEWIC 2011). Therefore, the recent population expansion of darters into the Grand River may be due to an unauthorized introduction, as was speculated for the greenside darter.

Among-river IBD patterns, determined for the Ohio River and Great Lakes drainages, suggest that, although within-river dispersal regulates the development of genetic structure, among-river hydrological distances can substantially influence genetic connectivity. As few among-river migrants were found throughout the species range, contemporary genetic structure is not strongly influenced by the among-river dispersal. No difference in IBD relationship was observed using littoral dispersal pathway distances rather than straight-line distances in the Great Lakes drainage (the only one with a large lake), suggesting that open-lake environments do not restrict movement; although, loss of suitable habitat availability in lakes accounts for declining lake populations (COSEWIC 2011). Population connectivity for this species is expected to be strongly impacted by anthropogenic barriers both within-river alteration of flows (e.g., dams or channelization) and modification of lacustrine shoreline habitats (e.g., dredging; Fisheries and Oceans Canada 2012). Therefore the already fragmented distribution of rivers occupied by eastern sand darter populations may be increasingly isolated by growing anthropogenic barriers.

Freshwater fish species inhabiting formerly glaciated regions commonly exhibit genetic signatures that reflect the influence of historical glacial refugia and re-

colonization patterns (Boizard *et al.* 2009; Stepien *et al*. 2007; Costello *et al*. 2003; Poissant *et al*. 2005; Shikano *et al.* 2010). Our study revealed that historic, post-glacial drainage patterns have an influence on large-scale (range-wide) genetic divergence patterns, contradicting contemporary drainage connectivity. The genetic separation of sites in the Ohio River drainage from the remainder of the species range is likely an artefact of the colonization of our sampled rivers in the Ohio River and Wabash River drainages from the Mississippi refugium following the most recent Wisconsinan glacial retreat (Underhill 1986). Therefore, re-colonization of the present-day rivers in the Ohio River likely occurred post-glaciation, and the separation of populations into the two drainages drives the genetic divergence observed between Ohio River and Wabash River drainages. Genetic similarity between the Wabash River and Great Lakes drainages likely reflects the historical connection of these two drainages at the end of the Wisconsinan glacial period (approximately 14 000 years ago), when excess water from the glacial Lake Maumee (ancestor of present-day Lake Erie) drained into what is now the Wabash River (Underhill 1986). The historic Maumee connection (also known as the "Fort Wayne" dispersal route) between the two drainages has been previously suggested to be a major dispersal corridor for aquatic organisms re-colonizing the Great Lakes (Underhill 1986, Mandrak & Crossman 1992), and to have driven genetic similarities between freshwater mussel (Bivalvia: Unionidae) populations in each drainage (Graf 2002; *Amblema plicata*, Elderkin *et al*. 2007).

Another important genetic influence of glacial colonization pathways on populations involves isolated, or "disjunct", species range patterns (Witt *et al.* 2011). A major genetic break identified in this study occurred between the St. Lawrence River

drainage and the remainder of the species range. Eastern sand darter is expected to have colonized Lake Champlain and the St. Lawrence River from the Mississippian glacial refugium through either the Mohawk River of the glacial Lake Iroquois (present-day Lake Ontario), 12 000-13 500 years ago, or through Lampsilis Lake (present day St. Lawrence River), 8 500-10 000 years ago (Underhill 1986). Both scenarios suggest that eastern sand darter should be present in Lake Ontario, and because none have been recorded, those populations may have experienced an undocumented extirpation or an alternate colonization route for the Lake Champlain population exists (COSEWIC 2011). Another hypothesis explaining the range disjunction may be that populations expanded into present-day Lake Ontario and the St. Lawrence River during the warm Hypsithermal Period (6,000 years ago) (Smith 1957); however, the subsequent cooling following this period may have extirpated Lake Ontario eastern sand darter populations but not the Lake Champlain or Lake Erie populations. The genetic discontinuity separating the St. Lawrence River drainage from the rest of the species range is especially important as sites within this region exhibited lower genetic diversity compared to sites from the rest of the range. The loss of population genetic diversity, coupled with higher levels of site genetic differentiation, is a common characteristic for isolated populations (Wofford *et al*. 2005). Eastern sand darter population in the St. Lawrence drainage experience genetic isolation and large geographic distance from the main species range providing evidence for the disjunction of the species range into two designatable units, as suggested by COSEWIC, as well as increased conservation concerns for these populations.

The northern range-edge eastern sand darter populations showed no evidence of loss of within-population genetic variation or anomalously high genetic divergence

contrary to the predicted results by the central-marginal range hypothesis (Lesica & Allendorf 1997). Previous studies of range-edge freshwater fish populations have demonstrated lower within-population genetic variation and higher among-population differentiation in fragmented range-edge populations (Beneteau *et al*. 2009; Zamudio *et al.* 2009). Also, colonization of drainages in formerly glaciated regions, particularly at species range-edges, can reduce genetic variability resulting from founder effects (Costello *et al*. 2003). The predominately low genetic structure across the eastern sand darter range reflects a combination of stratified dispersal and local extinction/colonization events resulting from unstable habitats. Lower genetic structure in the northern range-edge rivers reflects rapid population expansion and more recent colonisation than central range rivers, which can promote non-equilibrium genetic structure (Bronnenhuber 2011). Evidence for recent range expansion was present in the Grand River, although this may be human mediated. Finally, lower genetic differentiation in range-edge populations reflects unique population adaptations that permit increased dispersal capacity or behaviour (Roman & Darling 2007; Dytham 2009). However, our within-river dispersal analysis did not identify a higher number of migrants in the northern range-edge rivers compared to central range rivers.

Southern range-edge rivers were poorly represented in this study and no within-river comparisons could be made. Nevertheless, warming climates should promote extirpations along the southern range-edge resulting in a pole-ward shift in the species range (Chu *et al*. 2005). Therefore, our limited sampling success in the region, despite extensive sampling of known historical eastern sand darter habitat, indicates lower

species abundance in the region, which has serious conservation and management implications for the region.

Genetic analysis of population connectivity provides a quantitative characterization of population structure and insight into how gene flow impacts the structure and evolutionary processes within and among populations (Koizumi 2011). Our study emphasizes the blending of contemporary and historic influences on the genetic structure of eastern sand darter populations throughout the species range. The study highlights the influence of historic drainage connectivity and not only reveals genetic cohesiveness between previously connected drainages (e.g., the Wabash-Maumee historical connection) but also provides insight into the negative genetic effects of range isolation in "disjunct" drainages (e.g., St. Lawrence River drainage). Low genetic diversity and high among-site genetic differentiation in the St. Lawrence River drainage suggests that this drainage likely requires special management to maintain genetic diversity and, therefore, population viability. We found that range-edge genetic effects may not necessarily follow the predictions of the central-marginal range hypothesis, even though range-edge populations may exhibit unique genetic structure. Our range-edge populations exhibited lower genetic differentiation within rivers and also highlighted the potential for northern range-edge population expansions for the species, as demonstrated by Grand River. Our range-wide analysis of the genetic structure in a habitat-specific species clearly demonstrates that species-specific life history traits, such as dependence on specific habitat substrates, can strongly regulate genetic diversity patterns, likely through habitat stochasticity.

2.5 REFERENCES

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Table 2.1: Description of 39 eastern sand darter collection sites sampled in this study (see Fig. 1 for geographical locations). Site, letter code, GPS coordinate, number of individuals, corrected allelic richness (A_R), number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and Garza & Williamson "M" values are present. For F_{IS}, boldface type indicates significant result ($P < 0.05$).

Drainage	Site Name	Site ID	Latitude	Longitude	N	A_R	A	H_O	H_E	F_{IS}	M
Wabash R.											
	Eel R.	ER	40º49'41"	-86º06'50"	30	4.71	68	0.676	0.683	0.007	0.65
	East Fork White R.	EF	39º08'19"	-85º53'38"	32	5.53	91	0.694	0.747	0.073	0.75
	Big Creek	BC	38º48'33"	-85º38'38"	39	5.87	108	0.728	0.741	0.014	0.76
	Deer Creek	DC	39º30'02"	-86º55'49"	32	5.84	99	0.712	0.727	0.017	0.74
Ohio R.											
	Red R.	Rd	37º49'11"	-83º34'33"	17	5.31	69	0.714	0.777	**0.120**	0.73
	Licking R.	Lk	38º12'30"	-83º40'49"	19	5.33	74	0.580	0.687	0.010	0.80
	Salt Creek1	SC1	39º26'00"	-82º40'48"	16	5.42	72	0.704	0.700	-0.030	0.60
	Salt Creek2	SC2	39º20'59"	-82º40'40"	30	5.26	85	0.657	0.683	0.010	0.64
	Salt Creek3	SC3	39º19'50"	-82º40'56"	20	5.74	87	0.670	0.716	0.066	0.66
	Hocking R. main1	HRm1	39º18'03"	-81º57'50"	25	5.26	88	0.624	0.636	0.019	0.72
	Hocking R. main2	HRm2	39º17'44"	-81º56'14"	36	5.28	93	0.597	0.652	0.064	0.66
	Hocking R. main3	HRm3	39º17'48"	-81º54'05"	38	5.41	101	0.602	0.636	0.050	0.72
	Hocking R. creek1	HRc1	39º19'49"	-81º53'19"	37	5.67	113	0.664	0.662	-0.018	0.79
	Hocking R. creek2	HRc2	39º19'22"	-81º53'06"	28	5.50	96	0.640	0.654	-0.001	0.77
	Little Muskingum1	LM1	39º24'42"	-81º21'31"	17	5.55	75	0.769	0.719	-0.116	0.66
	Little Muskingum2	LM2	39º24'25"	-81º21'26"	38	5.63	101	0.683	0.677	-0.017	0.68
	Little Muskingum3	LM3	39º24'14"	-81º21'27"	24	5.78	93	0.676	0.688	-0.019	0.69
Great Lakes											
	St. Mary's R.	SM	40º53'41"	-85º00'26"	31	4.76	69	0.635	0.667	0.045	0.60
	St. Jospeph's R.	SJ	41º06'44"	-85º07'05"	35	5.05	77	0.654	0.710	0.077	0.63
	Maumee R. main1	MA1	41º05'03"	-85º01'11"	35	4.91	73	0.670	0.700	0.036	0.63
	Maumee R. main2	MA2	41º06'34"	-84º57'47"	32	4.92	76	0.675	0.691	0.013	0.64
	Maumee R. main3	MA3	41º07'50"	-84º56'06"	28	4.94	71	0.708	0.702	-0.010	0.63
	Sydenham	Syd	42º38'49"	-82º00'35"	12	5.47	68	0.600	0.702	0.135	0.71

	Thames R. upper1	THu1	42º55'55"	-81º25'35"	28	5.78	103	0.661	0.721	**0.085**	0.77
	Thames R. upper2	THu2	42º55'24"	-81º25'53"	27	5.58	93	0.640	0.708	**0.094**	0.71
	Thames R. upper3	THu3	42º54'30"	-81º25'30"	30	5.45	98	0.679	0.704	0.031	0.72
	Thames R. bigbend1	THd1	42º39'38"	-81º42'28"	32	5.60	99	0.741	0.727	-0.045	0.69
	Thames R. bigbend2	THd2	42º38'33"	-81º42'15"	24	5.30	84	0.730	0.712	-0.070	0.75
	Thames R. bigbend3	THd3	42º39'39"	-81º44'17"	21	5.66	88	0.757	0.736	-0.060	0.66
	Grand R. upper1	GRu1	43º07'40"	-80º11'57"	25	5.56	88	0.731	0.738	-0.011	0.66
	Grand R. upper2	GRu2	43º06'02"	-80º14'26"	17	5.26	77	0.694	0.726	0.045	0.72
	Grand R. upper3	GRu3	43º05'47"	-80º12'59"	27	5.49	88	0.740	0.747	-0.008	0.70
	Grand R. lower1	GRd1	42º59'04"	-79º52'25"	29	5.52	95	0.749	0.749	0.008	0.69
	Grand R. lower2	GRd2	42º58'15"	-79º52'48"	29	5.51	96	0.741	0.742	0.001	0.62
	Grand R. lower3	GRd3	42º57'31"	-79º52'12"	22	5.62	89	0.695	0.752	0.065	0.74
St. Lawrence R.											
	Rivière au Saumon	RAS	44º59'57"	-74º30'38"	21	4.26	61	0.631	0.621	-0.032	0.64
	Richelieu River1	RR1	45º38'06"	-73º11'26"	30	4.61	76	0.658	0.627	-0.062	0.72
	Richelieu River2	RR2	45º39'13"	-73º12'01"	27	3.94	62	0.560	0.570	0.003	0.67
	Champlain Canal	CC	43º21'09"	-73º29'44"	11	2.64	29	0.491	0.445	-0.108	0.60

Table 2.2: Within-river genetic differentiation among eastern sand darter sample sites from three different drainages (Ohio River, Great Lakes, and St. Lawrence River). Within each river, pairwise F_{ST} values (below diagonal) and pairwise chord distances, Dc values (above diagonal) were calculated among sites. Pairwise exact tests were also calculated, and significant results are indicated in bold above the diagonal.

Drainage							
Mississippi R.		LM1	LM2	LM3			
	LM1	*	**0.262**	0.288			
	LM2	0.007	*	0.226			
	LM3	0.003	-0.002	*			
		HRc1	HRc2	HRm1	HRm2	HRm3	
	HRc1	*	**0.239**	0.207	**0.246**	**0.218**	
	HRc2	0.009	*	**0.254**	**0.259**	**0.249**	
	HRm1	-0.003	**0.02**	*	0.236	0.217	
	HRm2	0.005	**0.021**	0.001	*	0.203	
	HRm3	0.003	**0.015**	-0.002	0.001	*	
		SC1	SC2	SC3			
	SC1	*	0.251	0.281			
	SC2	0.005	*	0.228			
	SC3	0.003	-0.003	*			
Great Lakes		THu1	THu2	THu3	THd1	THd2	THd3
	THu1	*	0.26	**0.248**	**0.248**	0.26	**0.282**
	THu2	0.004	*	**0.244**	**0.248**	**0.275**	**0.259**
	THu3	0.003	0.015	*	0.237	0.25	0.24
	THd1	0.003	0.005	0.002	*	0.245	0.251
	THd2	0.001	0.009	0	0	*	0.26
	THd3	0.008	0.006	-0.003	-0.002	-0.007	*
		GRu1	GRu2	GRu3	GRL1	GRL2	GRL3
	GRu1	*	0.29	**0.245**	**0.275**	**0.265**	**0.278**
	GRu2	-0.006	*	0.256	0.266	0.263	0.287
	GRu3	0.005	-0.005	*	0.257	0.249	0.235
	GRL1	0.009	-0.002	-0.002	*	**0.264**	0.246
	GRL2	0.004	-0.005	-0.004	0.001	*	0.256
	GRL3	0.005	-0.002	-0.008	0.002	-0.005	*

		SJ	MA1	MA2	MA3	SM
	SJ	*	**0.181**	**0.189**	**0.193**	**0.189**
	MA1	0.001	*	0.164	0.167	**0.171**
	MA2	0.001	-0.001	*	**0.206**	**0.192**
	MA3	0.007	0	0.012	*	**0.212**
	SM	0.012	0.009	**0.014**	**0.024**	*
St. Lawrence R.		RR1	RR2			
	RR1	*	0.212			
	RR2	0.005	*			

Bold indicates significance following Bonferroni correction (P < 0.01, 0.005, 0.01, 0.003, 0.003, 0.005, 0.05) below diagonal
Bold indicates significant pairwise exact test (P < 0.05) above diagonal

Table 2.3: Summary of genotype assignment results for all eastern sand darter sampled using GENECLASS. Individuals were considered successfully assigned as migrants when the assignment likelihood was > 0.10 and the rank-based method for highest likelihood assignment value to second highest likelihood assignment was higher than 4:1. Bold migrants indicate among drainage dispersals that are not likely due to natural dispersal.

River	N	Successful assignment	Migrants	Migrant source
ER	30	28	0	-
EF	32	23	0	-
BC	39	20	1	EF (1)
DC	32	20	1	ER (1)
Rd	17	15	0	-
Lk	19	19	0	-
SC	66	62	0	-
HR	164	148	6	LM (3), Rd (2), SC (1)
LM	79	67	2	**RR (1), RS (1)**
MA	161	130	0	-
Syd	12	11	0	-
TH	162	76	8	**RR (1), EF (2)**, MA (2), **ER (1), RS (2)**
GR	149	109	2	**HR (1),** TH (1)
RAS	21	18	0	0
RR	57	50	0	0
CC	11	11	0	0

Table 2.4: Migrants identified in two central range rivers (Hocking and Maumee) and two range-edge river (Thames and Grand) using GENECLASS, with individuals from each capture site (N) assigned using Bayesian individual assignment method (90% assignment threshold) of Rannala & Mountain (1997). Individuals successfully assigned to source sites using the rank-based method for highest assigned site to the second highest assigned site.

				Source site												
River	Capture site	N	Successful assignment	HRm1	HRm2	HRm3	HRc1	HRc2		SM	SJ	MA1	MA2	MA3		
Hocking R.																
	HRm1	25	14	14												
	HRm2	36	24		23	1										
	HRm3	38	15			15										
	HRc1	37	15	3		1	11									
	HRc2	28	20	1				19								
Maumee R.									-							
	SM	31	19							19						
	SJ	35	17								14	2		1		
	MA1	35	14							1		9	2	2		
	MA2	32	11									1	10			
	MA3	28	15								1			14		

				Source site												
	Capture site	N	Successful assignment	THu1	THu2	THu3	THd1	THd2	THd3		GRu1	GRu2	GRu3	GRd1	GRd2	GRd3
Thames R.																
	THu1	28	21	17	2		1	1								
	THu2	27	21		18	1		1	1							
	THu3	30	19	1	1	15	1		1							
	THd1	32	20				17	1	2							
	THd2	24	17					17								
	THd3	21	12						12							
Grand R.										-						
	GRu1	25	14								14					
	GRu2	17	13									13				
	GRu3	27	12										11		1	
	GRd1	29	15											14		1
	GRd2	29	16								1	1			14	
	GRd3	22	14											1		13

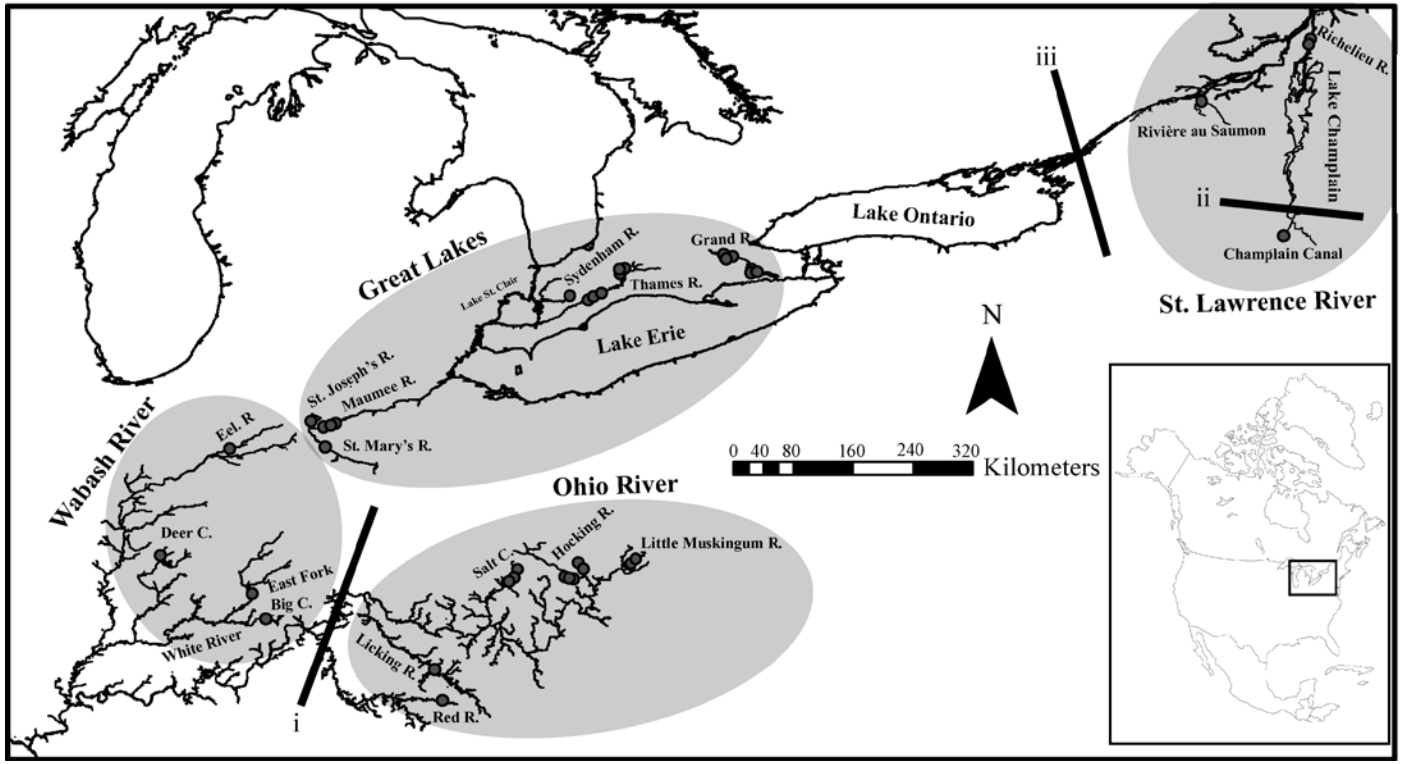


Figure 2.1: Eastern sand darter, *Ammocrypta pellucida*, collection sites (filled dots) across the species range in North America. Grey-shaded ellipses identify the four sampled drainages: Great Lakes drainage (Lake Erie/Lake St. Clair), Ohio River drainage, Wabash River drainage, and St. Lawrence River drainage (St. Lawrence River/Lake Champlain). Three major genetic discontinuities were identified across the species range using BARRIER software and are shown as black solid lines on the map, they represent; i) Ohio River separation, ii) St. Lawrence River isolation, iii) isolation of CC from the rest of the St. Lawrence River drainage.

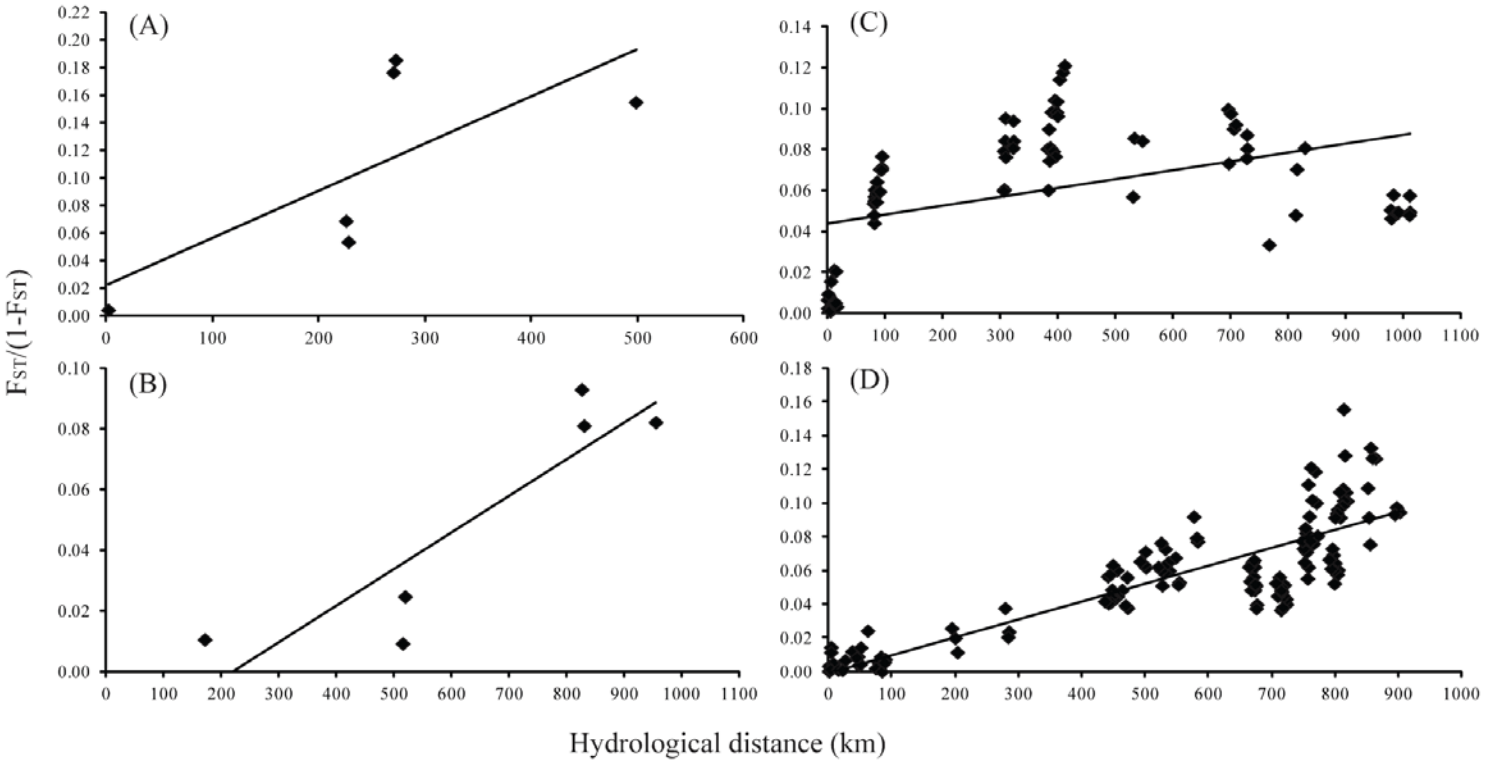
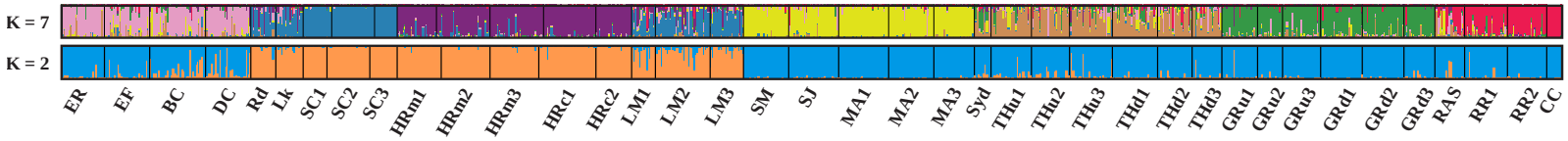


Figure 2.2: Isolation-by-distance relationship between linearized genetic differentiation [$F_{ST}/(1-F_{ST})$] and hydrological distance among eastern sand darter collection sites in the four drainages sampled. IBD relationship for; (A) St. Lawrence River drainage ($R^2 = 0.52$, $P = 0.084$) (B) Wabash River drainage ($R^2 = 0.79$, $P = 0.125$) (C) Ohio River drainage ($R^2 = 0.18$, $P < 0.004$) and (D) Great Lakes drainage (littoral distance, $R^2 = 0.80$. $P < 0.0001$). The strongest correlation between increased hydrologic distances and decreased gene flow among sites occurred for the Wabash River and Great Lakes drainages, although the only significant IBD equilibrium occurred in the Great Lakes and Ohio River drainages.

(A)



(B)

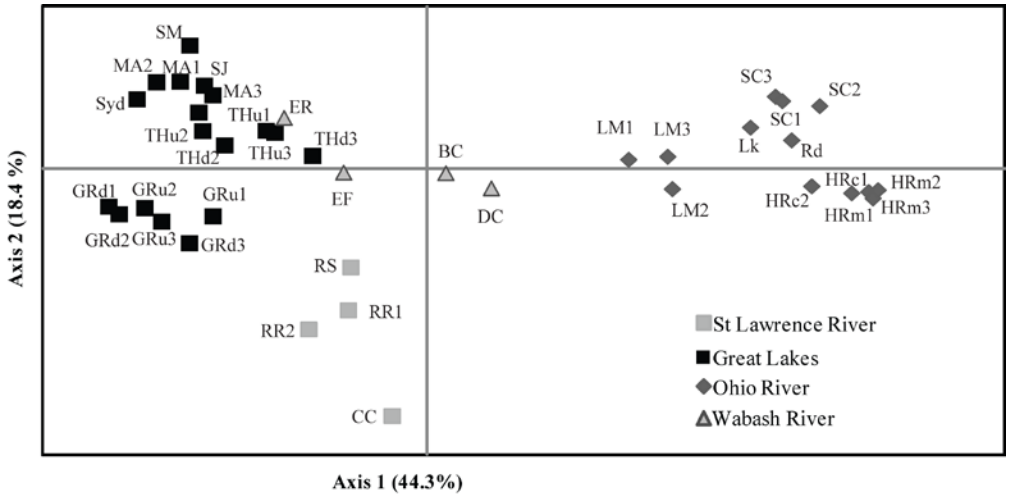


Figure 2.3: Range-wide genetic structure analysis showing (A) results of STRUCTURE analysis using 39 sample sites (see Table 1) across the eastern sand darter species range. STRUCTURE simulation summary for each sample site, with different colours showing each genetic cluster at K = 2 and K= 7 respectively. (B) Principle coordinate analysis (PCoA) performed using pairwise F_{ST} values among all sampled sites across the species range. The range was separated into 4 drainages; St. Lawrence River, Great Lakes, Ohio River, and Wabash River. The proportion of genetic variance explained by the first two axes is 62.7%.

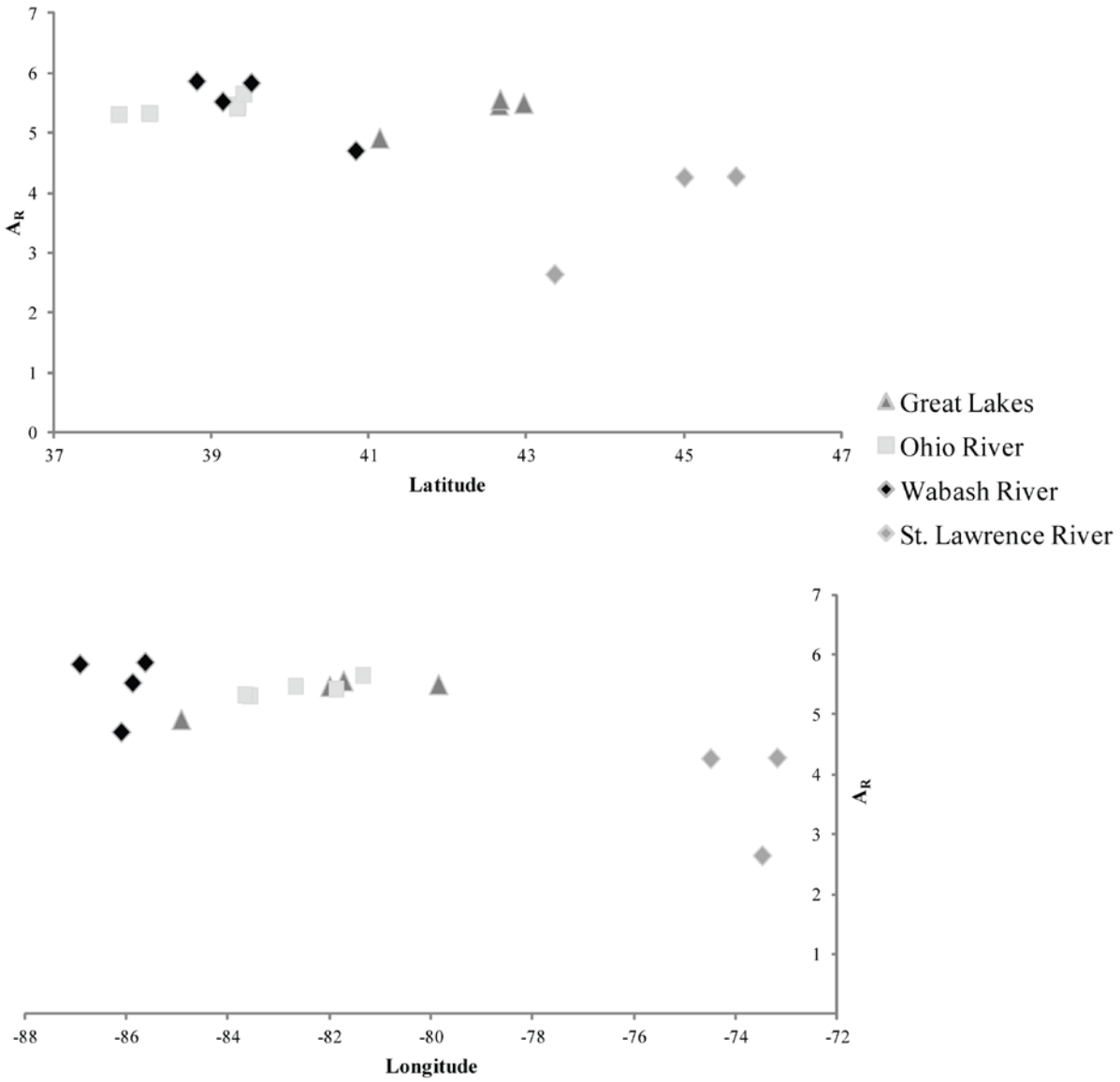


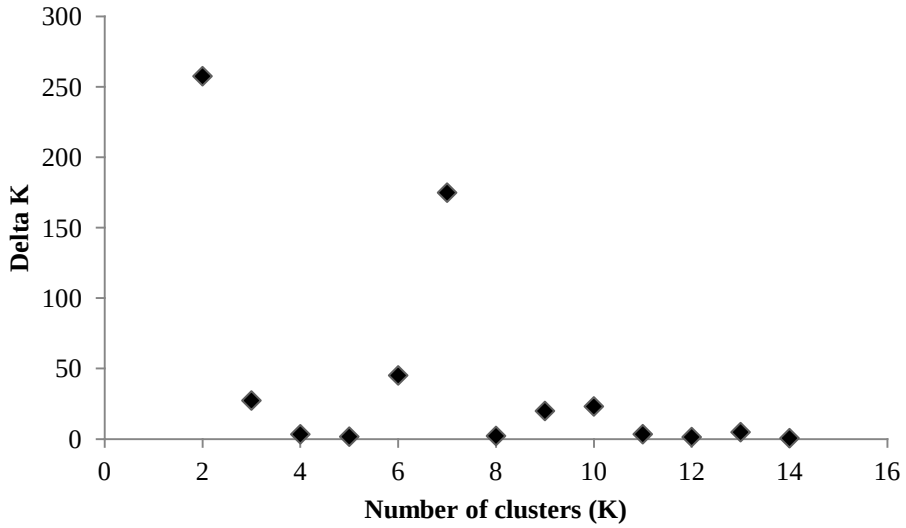
Figure 2.4: Range-wide comparison of eastern sand darter genetic diversity for 17 rivers, from the four drainages studied; Wabash River (ER, EF, BC, DC), Ohio River (Rd, Lk, SC, HR, LM), Great Lakes (MA, Syd, TH, GR), and St. Lawrence River (RAS, RR, CC). Genetic diversity was estimated by allelic richness (A_R) averaged across all sites within each river and compared against latitude and longitude values take from the within-river site closest to the river mouth.

Appendix 2.1: Characterization of ten microsatellite markers used for genetic analysis of *Ammocrypta pellucida*. GeneBank Accession numbers, primer sequences, repeat motif, optimal magnesium chloride concentrations, and annealing temperatures for each locus was determined. Allele frequency range and number of populations significantly deviating from HWE equilibrium following Bonferroni correction were calculated for each microsatellite.

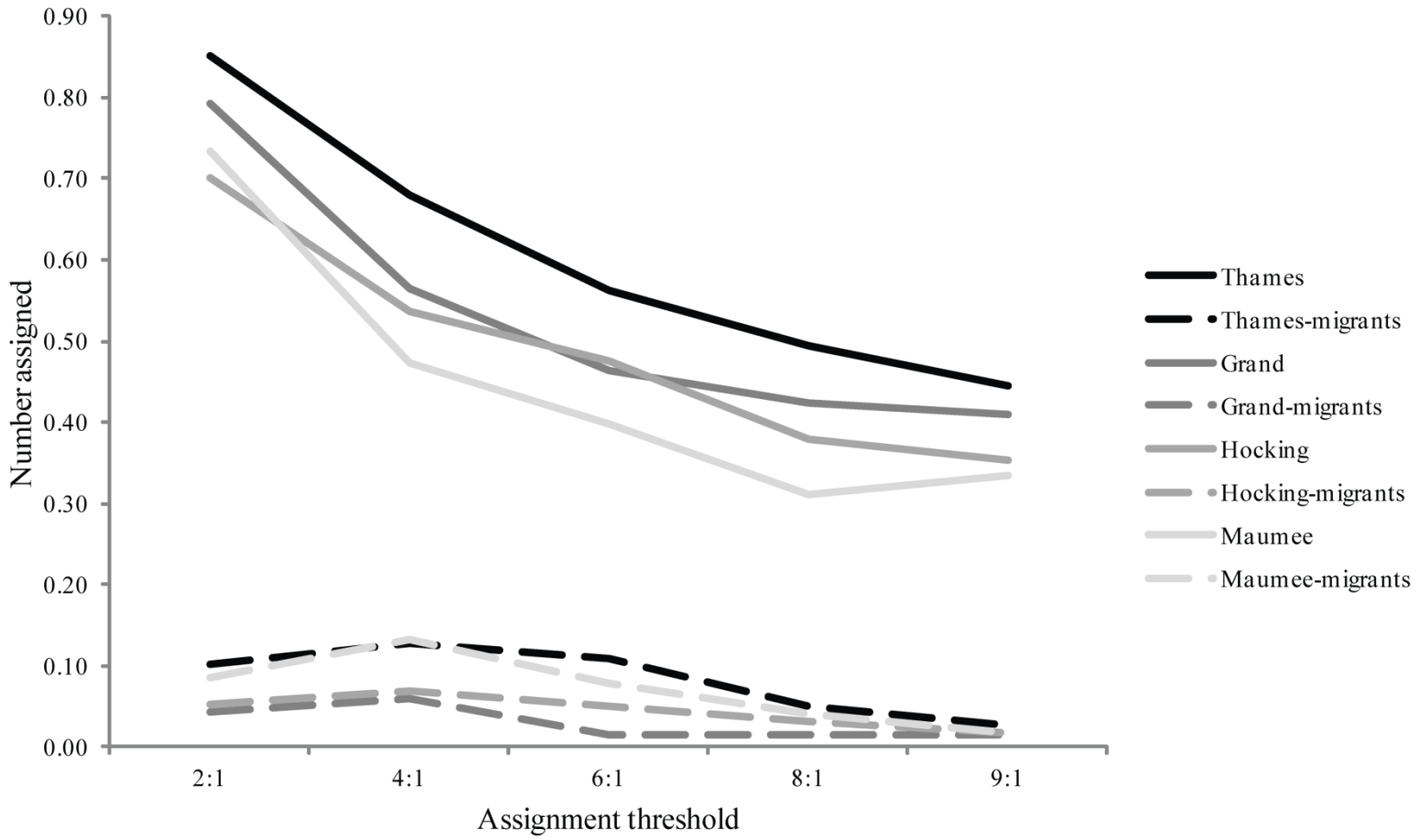
Locus	GenBank Accession	Primer sequence (5'-3')	Repeat motif	$MgCl_2$	Ta(^oC)	Allele Range (bp)	No. of alleles	HWE dev
EosC6	EF570435	F: AAAGCCTGAGGGACAATTACAC	$(CATC)_{13}$	2.2	58.0	265-349	12	0/40
		R: CCTTTGCTGGTAAATCTCACAC						
EosC112	EF570437	F: CATGCAGGTATGCACACGTA	$(AC)_{4...}(GGTA)_{11}$	2.2	58.0	165-181	8	3/40
		R: GGCAGTGGTGAGACAGAAAC						
EosD107	EF570444	F: CATTTAACATTCCCTGGTTGTG	$(TAGA)_{14}$	2.2	53.0	251-323	18	0/40
		R: TTGCAGTGCAGTGGAGTTTTA						
Esc132b	EF421255	F: GAAGCACCTCACCAAACAGCG	$(CTAT)_{33}$	2.2	56.6	144-212	18	0/40
		R: CCACACTGACACTGTGGACTGAC						
Esd3	HM775312	F: CAGCTGAGGTGTATACAAAACAAT	$(TC)_{17}$	2.1	59.5	172-214	13	0/40
		R: CAAAGCCTGCATGACAAAAA						
Esd17	HM775313	F: ACCCCCATCGGACTAATGTT	$(CA)_{12}$	2.1	58.2	142-342	70	1/40
		R: ATGTGTTGGTCCCTGAAAGC						
Esd18	HM775314	F: CCTGATGATTGAGATTGATGATG	$(GATA)_9(AC)_{12}$	2.1	55.0	173-251	37	0/40
		R: GAAGCACGCACATTCAGAAA						
Esd25	HM775315	F: TCATTCCACACCGTAACACG	$(CA)_{20}$	2.1	58.9	72-110	20	0/40
		R: TAGGACTGCCAGGTTGTGC						
Esd13	JQ439945	F: GTGGCTCCAAGATGCAAAGT	$(GT)_{15}$	2.1	61.0	127-163	9	2/40
		R: CCGCTCAGGGATCTAGTCTG						
EosD11	EF570443	F: ACCAGATGCAGTGGATGAATAT	$(TAGA)_{18}$	2.2	53.0	206-314	22	2/40
		R: GCGGTATCTAATGCTATTTCCC						

Appendix 2.2: Mean pairwise F_{ST} values below the diagonal and D_C values above calculated among each sampled river containing populations of easnter sand darter (*Ammocrypta pellucida*) from the 16 sampled rivers. Bold indicates significance following bonferroni correction ($P < 0.001$) for F_{ST} values and significance ($P < 0.05$) for D_C values.

	ER	EF	BC	DC	LK	RED	LM	HR	SC	MA	Syd	TH	GR	RAS	RR	CC
ER	*	**0.355**	**0.374**	**0.354**	**0.538**	**0.516**	**0.504**	**0.517**	**0.409**	**0.385**	**0.428**	**0.359**	**0.416**	**0.419**	**0.434**	**0.596**
EF	**0.075**	*	**0.274**	**0.303**	**0.508**	**0.488**	**0.519**	**0.475**	**0.380**	**0.324**	**0.419**	**0.317**	**0.387**	**0.382**	**0.370**	**0.543**
BC	**0.085**	*0.011*	*	**0.248**	**0.490**	**0.443**	**0.492**	**0.416**	**0.369**	**0.334**	**0.423**	**0.312**	**0.371**	**0.346**	**0.366**	**0.557**
DC	**0.076**	**0.024**	*0.009*	*	**0.470**	**0.442**	**0.479**	**0.423**	**0.352**	**0.344**	**0.429**	**0.320**	**0.364**	**0.382**	**0.359**	**0.568**
LK	**0.160**	**0.103**	**0.081**	**0.078**	*	**0.348**	**0.417**	**0.399**	**0.415**	**0.550**	**0.545**	**0.475**	**0.547**	**0.553**	**0.528**	**0.655**
RED	**0.144**	**0.089**	**0.069**	**0.063**	**0.032**	*	**0.397**	**0.387**	**0.387**	**0.493**	**0.533**	**0.444**	**0.497**	**0.526**	**0.487**	**0.626**
LM	**0.103**	**0.072**	**0.063**	**0.042**	**0.075**	**0.049**	*	**0.358**	**0.335**	**0.519**	**0.501**	**0.456**	**0.517**	**0.525**	**0.519**	**0.675**
HR	**0.164**	**0.119**	**0.085**	**0.073**	**0.080**	**0.046**	**0.053**	*	**0.308**	**0.499**	**0.502**	**0.420**	**0.480**	**0.490**	**0.471**	**0.610**
SC	**0.153**	**0.139**	**0.123**	**0.112**	**0.069**	**0.060**	**0.075**	**0.081**	*	**0.411**	**0.440**	**0.331**	**0.382**	**0.427**	**0.381**	**0.574**
MA	**0.081**	**0.047**	**0.058**	**0.077**	**0.148**	**0.145**	**0.120**	**0.165**	**0.162**	*	**0.406**	**0.307**	**0.378**	**0.377**	**0.366**	**0.581**
Syd	**0.062**	**0.071**	**0.084**	**0.084**	**0.172**	**0.159**	**0.121**	**0.175**	**0.154**	**0.054**	*	**0.315**	**0.372**	**0.437**	**0.452**	**0.614**
TH	**0.053**	**0.047**	**0.054**	**0.053**	**0.123**	**0.110**	**0.083**	**0.126**	**0.134**	**0.050**	*0.021*	*	**0.258**	**0.349**	**0.319**	**0.551**
GR	**0.099**	**0.077**	**0.090**	**0.088**	**0.156**	**0.149**	**0.109**	**0.168**	**0.165**	**0.090**	**0.044**	**0.055**	*	**0.404**	**0.334**	**0.558**
RAS	**0.114**	**0.070**	**0.056**	**0.060**	**0.159**	**0.147**	**0.115**	**0.130**	**0.171**	**0.096**	**0.116**	**0.081**	**0.105**	*	**0.305**	**0.474**
RR	**0.148**	**0.096**	**0.098**	**0.086**	**0.184**	**0.170**	**0.118**	**0.146**	**0.190**	**0.125**	**0.143**	**0.098**	**0.093**	**0.060**	*	**0.462**
CC	**0.259**	**0.170**	**0.184**	**0.193**	**0.279**	**0.267**	**0.224**	**0.237**	**0.281**	**0.243**	**0.289**	**0.204**	**0.205**	**0.155**	**0.175**	*



Appendix 2.3: Results of STRUCTURE analysis of the 39 sample sites, corresponding to Fig. 2.3, with Delta K (Evanno *et al.* 2005) calculated from the negative log likelihood [LnP(D)] provided by STRUCTURE, used to identify the true number of genetic clusters (K).



Appendix 2.4: Stringency analysis showing the effect of different assignment likelihood thresholds on the total number of fish assigned and the number of migrants assigned in GENECLASS.

3.0 RECOVERY STRATEGIES FOR THREATENED EASTERN SAND DARTER (*AMMOCRYPTA PELLUCIDA*) POPULATIONS; GENETIC INSIGHT AND RECOMMENDATIONS

3.1 INTRODUCTION

Analyzing populations from a variety of ecological, demographic, and genetic perspectives is important when developing recovery strategies for species in need of conservation (Koizumi *et al*. 2011). Species recovery actions may include; reintroduction of populations into previously extirpated regions of the native range, supplementation of existent populations using non-endangered populations, and introduction, which is less frequently used as it introduces populations into new regions when the historic range environments are being severely impacted (Armstrong & Seddon 2007; IUCN). Historically, population reintroduction and supplementation programmes have had limited success in establishing or maintaining viable populations (Fischer & Lindenmayer 2000). To improve the success of such programs, a more integrated approach for assessing the potential for reintroduction success by analyzing factors at the population, metapopulation, and ecosystem levels has been proposed (Armstrong & Seddon 2007). Recovery programs must include the multiple spatial scale analyses of demographic, ecological, and genetic impacts that these programs will have on the recipient populations and ecosystems, but also their influence on source populations. This new integrated approach highlights the need for multiple disciplines to act together to develop effective population recovery strategies. Species recovery programs often include translocations of individuals from non-threatened source populations, and/or captive breeding source populations, to supplement small or declining populations. The successful

implementation of such programs must include the genetic characterization of the source populations to avoid genetic deterioration of recipient populations through founder effects or outbreeding depression (Hedrick 1995; Huff *et al.* 2010).

Preservation of natural patterns of population connectivity is vital for the long-term success of recovery strategies, and genetic methods are useful for identifying the appropriate spatial scales for the implementation of conservation strategies (Austin *et al*. 2011). While monitoring ecological factors (natural and/or anthropogenic) and demographic processes (population growth and reproduction rates) is critical to determine basic population parameters for assessing population viability, a knowledge of the spatial genetic structure among populations helps determine contemporary and historic evolutionary influences on population connectivity (Wiens 1997; Lowe & Allendorf 2010). Identifying natural dispersal patterns can provide insight into demographic (e.g., population size and natural recovery potential) and genetic (e.g., gene flow) connectivity. Native population genetic structure can be used to determine the most appropriate pattern of introduction into the recipient habitat to ensure connectivity can persist in the recovering population (Armstrong & Seddon 2007). Gene flow among newly founded (or supplemented) populations will counteract genetic drift and thus minimize the need for additional, or ongoing, population supplementation (vonHoldt *et al*. 2010). Furthermore, high levels of gene flow within the recipient habitat will maintain large effective population sizes and high genetic diversity preserving evolutionary potential, valuable in the event of future environmental change (Hughes *et al.* 2008). Direct quantification of dispersal patterns using mark-recapture methods is difficult for many species, especially endangered species, as these methods are time-consuming, may fail to detect dispersals

beyond the sampled populations, and may not distinguish between instantaneous and effective dispersal (Schweizer *et al*. 2007). As such, indirect measures of dispersal using molecular genetic analyses can be used to quantify migration and gene flow, both current and historic (Chapter 2; Wilson *et al.* 2004; Schweizer *et al*. 2007). The identification of discontinuities in gene flow and subsequent genetic isolation can also provide insight into specific populations that are at the greatest conservation risk (Allendorf & Luikart 2007; Blouin *et al.* 2010).

Range expansions are difficult to characterize because they can result from natural or anthropogenic mechanisms, and both require a different resource management response strategies (Gozlan *et al*. 2010). Natural population introductions (i.e., species range expansions) often result in a stepping-stone establishment of populations in an area with genetic signatures reflecting the recurring founder effects within these populations (Dlugosh & Parker 2008; Wilson *et al*. 2009). However, when multiple unnatural introductions are unplanned (e.g., bait bucket transfer) but occur in the same area, or are planned (e.g., species translocation), then the loss of genetic diversity associated with founder effects may be reduced or eliminated (Beneteau *et al*. 2012). Furthermore, some species possess unique life history characteristics (e.g., "stratified" dispersal patterns or high propagule pools) that can facilitate rapid population expansions in introduced regions, thus allowing populations to preserve genetic diversity (Roman & Darling 2007; Bronnenhuber *et al*. 2011).

The eastern sand darter (*Ammocrypta pellucida*) is a benthic fish dependent on fine, sandy substrate habitats primarily in rivers. Such habitats are typically found on the depositional sides of river bends and fragmented by hundreds of meters of unsuitable

habitat within rivers (COSEWIC 2011). The sand bar habitat is susceptible to degradation from river flow alterations, especially those increasing siltation or those promoting channelization and elimination of sand deposition (COSEWIC 2011). Southern Ontario rivers have experienced a variety of anthropogenic impacts such as agriculture, urbanization, and construction of physical barriers in the river channels, and these are expected to strongly alter suitable substrate availability (COSEWIC 2011). Eastern sand darter spawn multiple times throughout the summer within a single year, and spawning occurs after the 1+ life-stage (Finch 2009; COSEWIC 2011). Average fecundity for a female eastern sand darter is 343 total ova, while mature ova range between 30-170 (Spreitzer 1979; Finch 2009).

Currently, eastern sand darter is listed as Threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and under Schedule 1 of the Canadian Species at Risk Act (SARA) and, therefore, requires the development of a recovery strategy and subsequent action plans (Fisheries and Oceans Canada 2012). A major factor in the species' decline in Canada is the deterioration of preferred habitat by anthropogenic influences, and in Ontario, only the Grand and Thames Rivers have stable populations (Fisheries and Oceans Canada 2012). Individuals in the Thames River have been repeatedly captured since its first collection in 1923, while Grand River population was not discovered until 1987, suggesting that this river may have been recently colonized (COSEWIC 2011). However, historic sampling did not specifically target eastern sand darter habitat, so the Grand River populations may represent previously undetected native species range (Fisheries and Oceans Canada 2012). Population extirpation has occurred in other southern Ontario rivers, such as: Big Otter Creek and

Catfish Creek in the Lake Erie drainage and Ausable River in the Lake Huron drainage (COSEWIC 2011), and the development of reintroduction programs has been identified as an important option for restoring eastern sand darter populations to their former range (Fisheries and Oceans Canada 2012). Furthermore, supplementation strategies may be appropriate within the declining eastern sand darter populations in Sydenham River in the Lake St. Clair drainage and Big Creek in the Lake Erie drainage (Fisheries and Oceans Canada 2012).

This study aims to characterize current population genetic viability and identify genetic connectivity patterns of eastern sand darter in two southwestern Ontario rivers to provide insight into future recovery strategies for these and other endangered eastern sand darter populations. Specifically, we propose recovery actions that are designed to retain genetic diversity in eastern sand darter populations currently considered at threat of extirpation. Specific objectives of this study are: i) to assess effective eastern sand darter population size in two threatened rivers and compare them to stable populations in Ohio and Indiana, ii) to identify within-river population connectivity in two southwestern Ontario rivers and provide suggestions for the re-colonization potential of other extirpated eastern sand darter populations in Ontario; and, iii) to test the hypothesis that the eastern sand darter populations in the Grand River is the result of a recent introduction/colonization. Our data provide baseline genetic information for an initial investigation into the feasibility of population reintroduction or supplementation actions for eastern sand darter populations in southwestern Ontario that had been identified as essential in the short-term recovery objectives of the recovery strategy (Fisheries and Oceans Canada 2012).

3.2 MATERIALS AND METHODS

Sample collection: This study was part of a larger study aimed at determining the range-wide spatial and temporal genetic structure of eastern sand darter. The initial range-wide spatial genetic structure study in Chapter 2 was performed using six of the same sample sites being used in this study, however, this study includes a number of additional sample sites in the Thames and Grand Rivers, as well as temporal samples for both, to increase our understanding of the fine-scale genetic patterns of eastern sand darter in these rivers. Adult eastern sand darter were collected in two sampling years (2010 and 2011) in the Thames and Grand Rivers in Ontario, Canada (Fig. 3.1). Juvenile eastern sand darter were only collected in the Grand River in 2011 as these were unexpectedly collected during the adult sampling. As Grand River sampling was done using seining and trawling, we tested for capture method sampling biases in body sizes using SPSS. Sampling sites were confined to the depositional sides of river bends and individuals were caught using a bag seine net (dimensions: 1.8m x 3.7m wings with 0.64cm mesh and 1.8m x 1.8m x 1.8m bag with 0.32cm mesh) or by using a Missouri trawl specialized for benthic fish collection (J. Barnucz, pers. comm., Fisheries and Oceans Canada, Burlington, ON). Pelvic fin clips were collected from each individual for subsequent DNA analysis, with fin clips stored in 95% ethanol, and the collected fish were released unharmed after a short recovery period.

DNA extraction and genotyping: DNA was extracted from fin clips using the column plate-based extraction protocol of Elphinstone *et al.* (2003). Five microsatellite markers were developed specifically for eastern sand darter using the enriched microsatellite library protocol of Fisher and Bachman (1998) as described in Chapter 2. Additionally,

four microsatellite primers previously developed for *Etheostoma osburni* (EosC6, EosC112, EosD107 EosD11; Switzer *et al*. 2008) and another developed for *Etheostoma scotti* (Esc132b; Gabel *et al*. 2008) were optimized for eastern sand darter. PCR amplification of all ten microsatellite loci used in this study was performed following the same protocol as in Chapter 2. Briefly, total reaction volumes were 12.75µl and each contained approximately 50-100ng template DNA, 25µM of dye labelled forward primer, 0.5µM of reverse primer, 200µM of each dNTPs, varying concentrations of $MgCl_2$ (see Appendix 2.1), and 0.25U *Taq* DNA polymerase (Applied Biosystems, Foster City, USA) in a 1X PCR buffer. The thermal cycler profile was initial temperature at 94^{o}C for 120 seconds followed by 35 cycles of 94^{o}C for 30 seconds, various annealing temperatures for each primer (see Appendix 2.1) for 45s, 30s at 72^{o}C, and 90s at 72^{o}C final extension. Dye-labelled PCR products were visualized on a LiCor 4300 DNA analyzer (Li-COR Biosciences, Inc.) polyacrylamide gel with 3 out of 67 lanes containing manufacturers' size standard (50-350bp). To determine individual genotypes, Li-COR gels were scored using GENE IMAGIR 4.05 software (Scanalytics Inc.).

Current population viability:

Effective population sizes: Central to the application of conservation genetics analyses to real-world management is the identification of effective population size (N_E) as this provides valuable information on the vulnerability of a population to genetic fluctuations and loss of genetic variation associated with genetic drift (Palstra & Ruzzante 2008). In some cases, low N_E estimates may give rise to increased conservation concerns for a population because of the threat of negative genetic effects associated with inbreeding depression, even when census size (N) estimates suggest acceptable population size (Friar

et al. 2000; Alo & Turner 2005). However, understanding the variance in effective population size is especially important as it provides a description of the rate of genetic change in a population that can be attributed to genetic drift (Wang & Whitlock 2003). We compared the effective population size ($N_{E(W)}$; Waples 1989) and the standardized allelic variance ($\hat{F}$; Waples 1989) between the Grand and Thames River populations using the temporal moment based-F-statistics method in N_E ESTIMATOR (Ovenden *et al.* 2007). The low genetic structure identified within each river suggested extensive gene flow (Chapter 2) so N_E estimates were calculated using fish from all sites combined within each river. As eastern sand darter are sexually mature after one year but individuals can live up to 4+ years, determined for Thames River eastern sand darter (Finch 2009), we corrected for overlapping generations by multiplying $N_{E(W)}$ estimates by the mean generation time of one year for the species, since eastern sand darter reproduce after the first growing season. To determine the influence of fluctuating population size on allele variance at each site, we compared the $\hat{F}$ estimates to the temporal change in catch-per-unit effort (ΔCPUE) estimated between sampling years using a Spearman rank correlation in SPSS. ΔCPUE was used as a proxy for the change in population size at each site as CPUE estimates have been shown to accurately estimate census size. As we did not have temporal samples for the non-threatened populations in the Hocking River (Ohio) and Maumee River (Indiana), genotyped in Chapter 2, we estimated N_E for these rivers using a single-sample N_E estimate. To make N_E comparisons between non-threatened rivers (HR and MA from Chapter 2) and the 2010 sampling data from the Thames and Grand Rivers, we estimated N_E for each river using the single-sample

linkage disequilibrium method to determine $\hat{N}_E$ (Hill 1981), with a bias correction in the program LDNE (Waples & Do 2008).

Population connectivity

Genetic structure: To identify genetic structure within the Grand and Thames Rivers, genetic differentiation among sites was characterized using pairwise F_{ST} estimated in ARELQUIN and genetic chord distances (Dc; Cavalli-Sforza & Edwards 1967), which do not assume a mutation model (as F_{ST} values do), in POPULATIONS v 1.2.28 (Langella 2002). To evaluate whether the pairwise F_{ST} values were significantly different from zero, the bootstrap significance was corrected for multiple simultaneous tests using both Bonferroni correlation (Evanno *et al.* 2005) and the false discovery rate (Benjamini & Hochberg 1995). Exact tests of allele frequency distribution differences were calculated for all pairwise site combinations within each river using FSTAT (Raymond & Rousset 1995). Genetic structure patterns within each river were visualized with a principle coordinates analyses (PCoA) using pairwise F_{ST} values in GENALEX.

Dispersal: To identify within-river dispersal, we used the Bayesian genotype assignment method (Rannala & Mountain 1997) in GENECLASS 2.0 (Piry *et al.* 2004), with an assignment threshold of $P > 0.05$ independently for the Thames and Grand Rivers. For individuals that assigned to any site, we identified the most likely source site using the rank-based method in GENECLASS, with a threshold that the highest assigned probability must exceed 4 times the next highest likelihood assignment probability. This approach has been identified as robust based on a sensitivity analysis (Chapter 2). We classified each dispersal event as either upstream or downstream and calculated whether

the direction was biased using a Chi-squared (χ^2) test with the null hypothesis of no directional bias (1:1). Downstream dispersal bias is expected to create an upstream to downstream gradient of genetic diversity (i.e. lower genetic diversity upstream, and higher diversity downstream). To test this hypothesis, we used Pearson product moment correlations (r) to calculate the relationship between the distance from each sample site to the river mouth (km) and estimates of genetic diversity (H_E and A_R) in SPSS. A downstream bias in gene flow should result in a negative relationship between distance upstream and genetic diversity measures.

We also explored the potential for young-of-the-year dispersal (passive or active) to drive connectivity in the eastern sand darter by genotypically assigning 0+ age-class individuals (< 35mm) collected in the summer of 2011 to their source (parent) populations from 2010; however, because of limited success in capturing juveniles, this analysis was only performed for the Grand River. The eastern sand darter 0+ age-class fish were identified based on body size following Finch (2009).

Grand River introduction:

Genetic diversity: Recently established populations are not expected to exhibit significant genetic structure if the time-scale is too short for genetic drift to occur. Therefore, global F_{ST} values were calculated for the Grand River and the Thames River to determine the overall genetic differentiation within each river using FSTAT (Weir & Cockerham 1984), and compared with the expectation that if the Grand River eastern sand darter were recently introduced, they would exhibit lower genetic structure (F_{ST}). Lack of within-river genetic structure is also expected to restrict the development of isolation-by-

distance (IBD) as these patterns develop over time as a result of higher gene flow among geographically close sites. IBD was estimated as the linearized genetic differentiation ($F_{ST}/1\text{-}F_{ST}$) and compared to hydrological distances (km) among sites for each river, with significance determined using a Mantel test in GENALEX. We calculated IBD in the long-established Thames River population to verify that eastern sand darter populations will exhibit IBD over time, and compared those results to IBD results from the Grand River to test the recent introduction hypothesis.

Finally, if eastern sand darter were recently introduced to the Grand River, we expect to be able to identify a likely source population for the introduction. We thus used the Bayesian genotype exclusion method of Rannala and Mountain (1997) in GENECLASS to exclude possible sources for a putative Grand River eastern sand darter introduction (Beneteau *et al*. 2012). The genetic exclusion method used the Grand River eastern sand darter as "individuals to be assigned" and these were either excluded ($P < 0.10$), uncategorized ($0.10 < P < 0.90$), or "likely assigned" ($P > 0.90$) to the Thames River, Richelieu River (Quebec), Maumee River (Indiana), Hocking River (Ohio), or Salt Creek (Ohio) as potential sources (see Fig. 2.1).

3.3 RESULTS

Sampling : A total of 390 eastern sand darter were collected in the Thames River in 2010, while 273 were collected in 2011 (Table 3.1). In the Grand River, 377 individuals were collected in 2010, while 236 were collected in 2011 (Table 3.1). Of the Grand River individuals collected in 2011, 67/326 were juvenile young-of-the-year (Table 3.1). In 2010 sampling, 324 eastern sand darter were collected using a trawl and 53 were collected using a seine. A Mann Whitney U test found that there was a significant (P =

0.42) eastern sand darter size class collection bias for the two different sampling methods that were used as the trawl caught smaller eastern sand darter; however, these results should be taken with caution as we used the trawling technique much more often than the seining technique.

Genetic analysis:

Effective population size: Temporal effective population size estimates ($N_{E(W)}$) were higher in the Grand River ($N_{E(W)}$ = 452.0; 95% CI = 230.5-1414.2) compared to the Thames River ($N_{E(W)}$ = 257.6; 95% CI = 100.7-228.5), although not significantly different based on confidence interval overlap (Fig 3.2). $\hat{F}$ values for temporal allele variance were higher for the Thames River (0.0019) compared to the Grand River $\hat{F}$ estimate (0.0011). No significant correlation (rho = -0.14, P = 0.62) was determined between changes in catch-per-unit effort and temporal allele frequency variance for either river using the Spearman rank correlation, although some samples sites (GRu1, GRu4, GRu5, THd1, THd2, THd10) could not be included as $\hat{F}$ was estimated as infinite. LDNE comparisons to the non-threatened rivers revealed that the Thames and Grand Rivers had higher $\hat{N}_E$ estimates ($\hat{N}_E$ = 2910; 95% CI = 2400 -13400 and $\hat{N}_E$ = 2400; 95% CI = 1370- ∞, respectively) than that of the Hocking River ($\hat{N}_E$ = 307; 95% CI = 244-405), while the Maumee River had the highest estimate ($\hat{N}_E$ = 5400; 95% CI = 983- ∞) (Table 3.2).

Characterization of population connectivity:

Genetic structure: No significant genetic differentiation, following both Bonferroni and false discovery rate correction, was present among sample sites in 2010 in either river as F_{ST} values ranged between -0.008 and 0.022 in the Grand River and -0.007 and 0.015 in

the Thames River (Appendix 3.1). D_C values for the Thames River ranged between 0.195-0.276 while Grand River D_C values ranged between 0.189-0.308. Exact tests of allele frequency distribution differences found a higher proportion of significant pairwise site combinations in the Thames River (42.3%; 33/78) compared to those within the Grand River (19.7%; 13/66) (Appendix 3.1). Within the Thames River, 22 out of the 33 significant exact tests included the most upstream sites (THu1/THu2), while 9 out of the 13 significant exact tests in the Grand River included the GRu3 site (Appendix 3.1). PCoA corroborated the lack of spatial genetic structure within the Grand River; however, it also revealed the upper Thames River sites (THu1, THu2, and Thu3) were slightly divergent from the other Thames River sites (Fig. 3.2).

Dispersal: GENECLASS analyses revealed similar numbers of migrants within each river; 15.5% (24/155) of the assigned fish were identified as migrants in the Thames River, while 18.2% (25/137) of the assigned individuals were identified as migrants within the Grand River, at the 4:1 threshold for successful assignment (Table 3.3). No significant directionality was determined as dispersal patterns identified 13 individuals as upstream migrants and 11 individuals as downstream migrants in the Thames River (P = 0.69), while 15 upstream and 10 downstream migrants were identified in the Grand (P = 0.32) (Table 3.3). Dispersal distances did not appear to be limited by hydrologic distances in either river as the highest frequency of dispersing individuals occurred at > 30km in both rivers (Fig 3.3). We found that only 14.9% (10/67) of the sampled juveniles assigned to any site at the 4:1 ratio; however none of juveniles were genetically assigned to their site of capture. Of the juvenile migrants, 7 individuals were identified as downstream dispersals and 3 as upstream dispersals, not significantly different from the

null expectation of random dispersal (P = 0.21). When the assignment threshold was relaxed to 3:1, again no juveniles were self-assigned and of the 16 identified juvenile migrants, 12 were downstream migrants compared to 4 upstream migrants representing a significant downstream dispersal bias for juveniles (P = 0.0015).

Grand River introduction:

Global F_{ST} values were extremely low for both of the rivers, with the Thames River $F_{ST} = 0.003 \pm 0.001$, and the Grand River $F_{ST} = 0.001 \pm 0.001$. The Isolation-by-distance correlation between linearized F_{ST} values and hydrological distances between sites revealed a significant IBD pattern within the Thames River ($R^2 = 0.22$, P = 0.012) while the Grand River had no IBD correlation ($R^2 = 0.0065$, P = 0.427) based on the Mantel test (Fig. 3.4). We note that the IBD analysis will be influenced by non-continuous pairwise sampling in each river, which resulted in the exclusions of intermediate pairwise hydrological distances for each. Within the Grand River, pairwise hydrological distances between 23-32km were not included because of the presence of a dam separating the upstream and downstream sites. Within the Thames River, pairwise hydrological distances between 32-58km were not included because of sampling regulations in the stretch of river separating upstream and downstream sites. Genotype assignment of the Grand River samples to potential source rivers identified that the Richelieu, Maumee, Hocking and Salt Creek Rivers could be excluded as potential source rivers for the Grand River populations as 95% (359/377), 99.5% (357/377), 99.7% (1/377), and 99.7% (1/377) were excluded (P < 0.10) for each river, respectively (Fig. 3.5). The GENECLASS exclusion analysis does not exclude the Thames River as 88.6%

(334/377) of the individuals did not exclude the Thames River as a potential source ($P > 0.10$), however, only 1.1% (4/377) of fish were assigned ($P > 0.90$) (Fig. 3.5).

3.4 DISCUSSION

Comparisons of $\hat{N}_E$, estimated using the linkage disequilibrium method, between the threatened eastern sand darter populations in southwestern Ontario (Thames and Grand Rivers) to the non-threatened populations in Ohio and Indiana revealed that neither Canadian river exhibited dramatically lower $\hat{N}_E$. Range-wide genetic diversity estimates from Chapter 2 corroborate our $\hat{N}_E$ results, as no loss of allelic richness or heterozygosity was found for eastern sand darter populations considered threatened in Ontario. Rather, here we show that the Hocking River eastern sand darter populations may be at higher risk for loss of genetic diversity and inbreeding depression associated with low effective population size. Our $\hat{N}_E$ estimates indicate that eastern sand darter populations in both sampled Canadian rivers have relatively high effective population sizes to maintain neutral genetic variance over evolutionary time scales ($N_E > 500$; Franklin & Frankham 1998). However, our temporal estimates of N_E ($N_{E(W)}$) suggest that temporal allele frequency changes resulted in N_E values that are lower than those expected for a viable population ($N_E < 500$) (Franklin & Frankham 1998; Johnson *et al*. 2004). Temporal models for determining N_E assume that all changes in allele frequencies are a result of genetic drift. Thus, if other population demographic factors contribute to changes in allele frequency distributions over time, then $N_{E(W)}$ will be biased downward. Our $\hat{F}$ estimates, measuring allele frequency variance over time, found that values determined for Grand and Thames Rivers as a whole were lower than those determined for individual sites within the rivers, indicating higher genetic instability at individual sites rather than the

entire river. A possible mechanism that could explain the pattern of temporal genetic variation in the Grand and Thames Rivers is the differential survival and colonization of sub-populations (Walter *et al*. 2009). This could be mediated by the extreme dependence of eastern sand darter on potentially unstable sand bar habitats. Evidence for population extinction/re-colonization or, more accurately, population re-location/re-colonization, was provided by the observed differences in the CPUE from 2010 and 2011 at multiple sites in both sampled rivers (e.g., Thd3, 11GRn1, and GRu1). Another study examining eastern sand darter CPUE found yearly fluctuations over a five-year period, and those fluctuations were attributed to unstable habitats, although variable reproductive success was also suggested as a possible factor (Facey 1998). Substantial annual variation in average CPUE was also demonstrated previously for eastern sand darter in the lower Thames River (Finch 2009). Therefore, extinction/re-colonization of unstable sand bar habitat patches may be a common demographic characteristic for eastern sand darter populations. Previous studies have demonstrated a relationship between temporal genetic instability (and reduced N_E) and unstable geological environments (Ostergaard *et al*. 2003; Shrimpton & Heath 2003).

Effective population size and genetic diversity comparisons to non-threatened populations (Ohio/Indiana) indicate that the Grand and Thames River populations are stable and do not require immediate direct conservation intervention to address loss of genetic diversity. However, these two rivers may be useful candidates as potential donor populations for other rivers in southwestern Ontario that currently have low and declining population sizes (e.g., Sydenham, Big Creek) or are already extirpated (e.g., Ausable River, Big Otter Creek, Catfish Creek) (Fisheries and Oceans Canada 2012). Potentially

extant recipient populations need to be genetically characterized to verify that the genetic variation introduced from potential source populations will not genetically swamp locally adapted populations and thus lead to outbreeding depression (Bouzat *et al*. 2009). In Chapter 2 we found low levels of genetic differentiation between eastern sand darter populations in the Sydenham and Thames Rivers, which is encouraging for potential supplementation of the declining populations in the Sydenham River from populations in the Thames River.

Maintenance of genetic variability within sample sites and low genetic differentiation among sites can be attributed to the "stratified" dispersal patterns in the Grand and Thames Rivers (Bronnenhuber *et al*. 2011). The high dispersal and subsequent gene flow within rivers has two major implications for reintroduction strategies: i) rapid colonization of the recipient river; and, ii) maintenance of gene flow to sustain future genetic variability (Hedrick & Frederickson 2010). Rapid population expansion in the recipient rivers following a population introduction is a useful trait for eastern sand darter as it will reduce the loss of genetic diversity associated with small population founder effects (Friar *et al*. 2000; Roman & Darling 2007). High gene flow among populations in the Grand and Thames Rivers suggests that genetic drift and, ultimately, inbreeding depression, will be minimized in reintroduced populations if these rivers were used as source populations, and they retain their dispersal characteristics (Friar *et al*. 2000). Another strategy that can be implemented to ensure the preservation of genetic diversity in reintroduced populations is the use of multiple introductions in the recipient river as these can maintain high effective population sizes, thus, minimizing effects of genetic drift in populations (Roman & Darling 2007; Beneteau *et al*. 2012). However, the

significant IBD present in the Thames River suggests that gene flow is restricted when populations are separated by greater than 60 km; therefore, allocation of multiple Thames River populations into recipient rivers should not likely exceed 60 km to ensure genetic connectivity of populations.

The anomalously low genetic structure identified in eastern sand darter sites in the Grand River, relative to the longer-established Thames River, supports the hypothesis that eastern sand darter were recently introduced into the Grand River. In all southwestern Ontario rivers that have been identified as harbouring eastern sand darter, the only river where sampling records do not identify eastern sand darter collections prior to 1970 is the Grand River, where individuals were not first recorded until 1987 despite previous fish surveys in the river (COSEWIC 2011). In areas with long colonization histories, gene flow is expected to be constrained among geographically distant sites and IBD equilibrium should emerge (Lowe & Allendorf 2010). The Thames River demonstrated this pattern; however, in the Grand River, despite the separation of sites by up to 60 km, as well as a large physical impoundment at the Caledonia dam, minimal genetic differentiation among site precluded IBD. As Grand River eastern sand darter populations are recently introduced, we identified the Thames River, which was also identified for the greenside darter Grand River introduction (Beneteau *et al*. 2012), as the most likely colonization source for the Grand River populations using our genotype assignment analysis (Fig. 3.5). However, because significant genetic differentiation persists among the Thames and Grand Rivers (Chapter 2), the Grand River populations may have originated from another river that was not sampled in our study. A potential route for the introduction of eastern sand darter from the Thames River to the Grand

River could have been through large-scale fish transfers of walleye (*Sander vitreus*) that occurred in the mid-1980s (MacDougall *et al*. 2007). We also note an emerging pattern of low within-river population genetic structure and maintenance of genetic diversity for a variety of variety of fish species in the Grand River (e.g., greenside darter, Beneteau *et al*. 2012; black redhorse, *Moxostoma duquesnei*, Reid *et al*. 2008), despite extensive physical impoundments (e.g., dams) in the river (Southam *et al*. 1999). We suggest that this pattern should be further explored using other fish species to identify potential river dynamics or historic and/or current river management practices that maintain extensive population connectivity for fish populations in the Grand River.

Preservation of suitable sand-bar habitats is highlighted in the eastern sand darter species recovery strategy (Fisheries and Oceans Canada 2012). Our genetic structure analyses support this given that eastern sand darter populations appear to experience frequent extinction/re-colonization, within rivers. Thus, rather than focussing on the conservation of a single sand-bar population within a river (which may not be temporally stable), conservation actions should be aimed at preserving both inhabited and uninhabited sand bars. Support for this approach was found in a recent study by Tessler *et al.* (2012), which that demonstrated that changes in Maumee River drainage agricultural practices has facilitated the re-colonization of eastern sand darter populations into stretches of the river where they had been previously extirpated due to siltation. As the eastern sand darter populations in the Thames River have retained genetic diversity, coupled with apparently high dispersal capacity, the Thames River populations represent a viable population. Eastern sand darter populations in the Grand River appear to have resulted from a recent introduction and these populations have since experienced a

subsequent increase in population size; therefore, Grand River eastern sand darter populations are also not likely under serious threat of extirpation. The recent successful introduction of eastern sand darter populations in the Grand River suggests that the species may be a good candidate for reintroduction recovery actions. We also suggest that the Thames River populations are likely the most appropriate source populations for reintroduction into other southwestern Ontario rivers where eastern sand darter populations have been extirpated.

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Table 3.1: Description of 26 eastern sand darter sample sites in the Thames and Grand Rivers in Ontario. Site name, GPS coordinate, number of individuals collected in 2010 (N_{2010}), number of individuals collected in 2011 (N_{2011}), number of juveniles ($N_{juv2011}$), corrected allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and distance in kilometers to river mouth (rkm) are shown for each site.

River	Site Name	Latitude	Longitude	N_{2010}	N_{2011}	$N_{juv2011}$	A_R	H_O	H_E	rkm
Thames R.										
	Thames upstream1	42º55'55"	-81º25'35"	28	37		5.80	0.664	0.724	184.0
	Thames upstream2	42º55'24"	-81º25'53"	27	22		5.58	0.640	0.709	182.6
	Thames upstream3	42º54'30"	-81º25'30"	33	22		5.45	0.682	0.706	177.9
	Thames downstream1	42º42'29"	-81º36'59"	33	51		5.78	0.721	0.733	119.2
	Thames downstream2	42º41'52"	-81º39'21"	37	9		5.66	0.711	0.722	112.7
	Thames downstream3	42º41'36"	-81º41'11"	28	-		5.66	0.767	0.746	109.5
	Thames downstream4	42º40'23"	-81º41'27"	33	22		5.75	0.722	0.731	106.2
	Thames downstream5	42º39'38"	-81º42'28"	32	25		5.60	0.746	0.727	102.5
	Thames downstream6	42º38'33"	-81º42'15"	26	25		5.37	0.729	0.713	99.1
	Thames downstream7	42º38'26"	-81º42'08"	25	25		5.69	0.736	0.721	98.8
	Thames downstream8	42º39'08"	-81º43'19"	36	-		5.48	0.720	0.721	95.5
	Thames downstream9	42º39'39"	-81º44'17"	26	25		5.68	0.737	0.734	93.7
	Thames downstream10	42º38'09"	-81º46'41"	26	10		5.70	0.762	0.738	88.1
Grand R.										
	Grand upstream1	43º06'34"	-80º14'48"	58	10		5.47	0.731	0.737	93.8
	Grand upstream2	43º06'38"	-80º14'37"	38	-		5.24	0.729	0.727	93.5
	Grand upstream3	43º07'40"	-80º11'57"	26	23		5.66	0.735	0.742	84.0
	2011Grand new1	43º07'03"	-80º12'35"	-	25		-	-	-	81.7
	Grand upstream4	43º06'28"	-80º13'43"	29	20		5.37	0.722	0.739	80.5
	Grand upstream5	43º06'02"	-80º14'26"	17	12		5.26	0.694	0.727	78.9
	Grand upstream6	43º05'47"	-80º12'59"	31	27		5.52	0.732	0.748	76.2
	Grand upstream7	43º05'52"	-80º12'52"	24	22	2	5.11	0.747	0.709	75.9
	Grand upstream8	43º05'31"	-80º11'09"	45	21	11	5.28	0.734	0.729	73.4
	Grand upstream9	43º06'19"	-80º07'46"	16	24	20	4.99	0.700	0.698	71.0
	Grand downstream1	42º59'05"	-79º52'20"	29	28	21	5.59	0.748	0.752	37.1
	Grand downstream2	42º58'15"	-79º52'48"	29	24	13	5.51	0.741	0.742	35.3
	Grand downstream3	42º57'31"	-79º52'12"	35	-	-	5.59	0.711	0.744	33.5

Table 3.2: Estimates of the effective population sizes using three different methods; the temporal $N_{E(W)}$ estimate of Waples (1989) and the bias corrected linkage disequilibrium $\hat{N}_E$ estimate of Waples & Do (2008).

	Temporal method			Linkage disequilibrium method		
River	$N_{E(W)}$	lower 95%	upper 95%	$\hat{N}_E$	lower 95%	upper 95%
Grand River	452.0	221.5	1866.2	2912.8	1365.9	∞
Thames River	257.6	156.9	486.1	2403.0	1281.0	13425.3
Maumee River	-	-	-	5422.6	983.1	∞
Hocking River	-	-	-	306.8	243.8	405.1

Table 3.3: Migrant eastern sand darter identified in the Thames and Grand Rivers using GENECLASS. Individuals captured in 2010 (N) were assigned using Bayesian individual assignment method (90% assignment threshold) of Rannala & Mountain (1997). Individuals successfully assigned (N_{assign}) were subsequently assigned to a specific source site if the ratio of the highest assignment likelihood to the second highest likelihood exceeded 4, using the rank-based method.

			Source site												
Collection site	N	N(assigned)	THu1	THu2	THu3	THd1	THd2	THd3	THd4	THd5	THd6	THd7	THd8	THd9	THd10
THu1	28	14	12	1							1				
THu2	27	18		16								1			1
THu3	33	14	1		10					1			1	1	
THd1	33	12				10					1		1		
THd2	37	10		3			7								
THd3	28	13		1				10	1		1				
THd4	33	13							11		1		1		
THd5	32	12						1	1	10					
THd6	26	11									11				
THd7	25	7					1					6			
THd8	36	13	1									1	11		
THd9	26	9								1				8	
THd10	26	9													9

			Source site											
Collection site	N	N > 4:1	GRu1	GRu2	GRu3	GRu4	GRu5	GRu6	GRu7	GRu8	GRu9	GRd1	GRd2	GRd3
GRu1	58	16	10	1				1	3				1	
GRu2	38	12		10					1	1				
GRu3	26	16			14						1		1	
GRu4	29	7				6	1							
GRu5	17	6					6							
GRu6	31	12						10					2	
GRu7	24	15	1						13		1			
GRu8	45	12		2		1				8	1			
GRu9	16	12									12			
GRd1	29	13			1				1			11		
GRd2	29	9				1							8	
GRd3	35	7								1	2			4

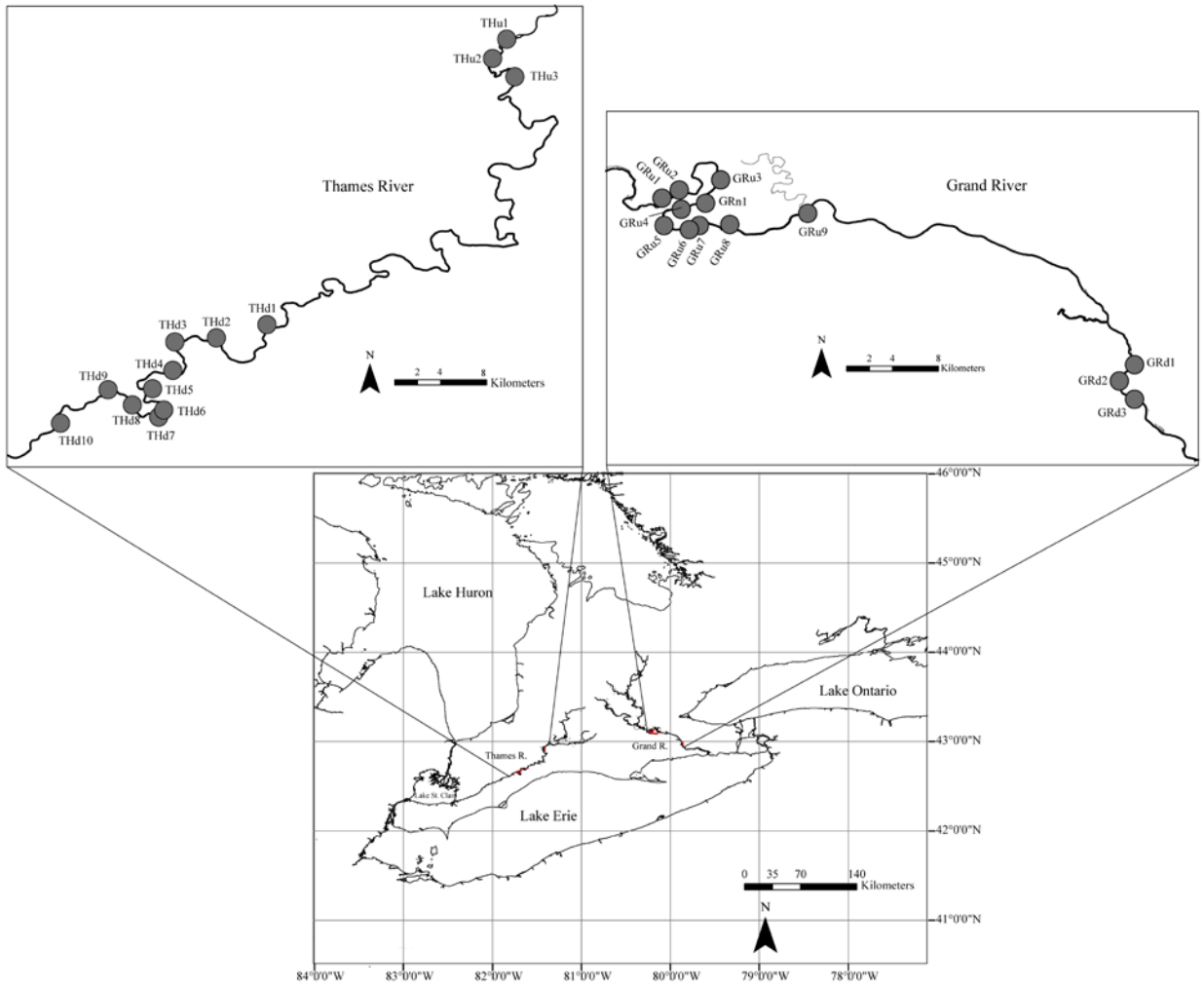

Figure 3.1: Eastern sand darter collection sites (see Table 3.1 for site codes) in two southwestern Ontario rivers, the Thames River and Grand River. We collected a total of 1276 fish over two years of sampling for the study.

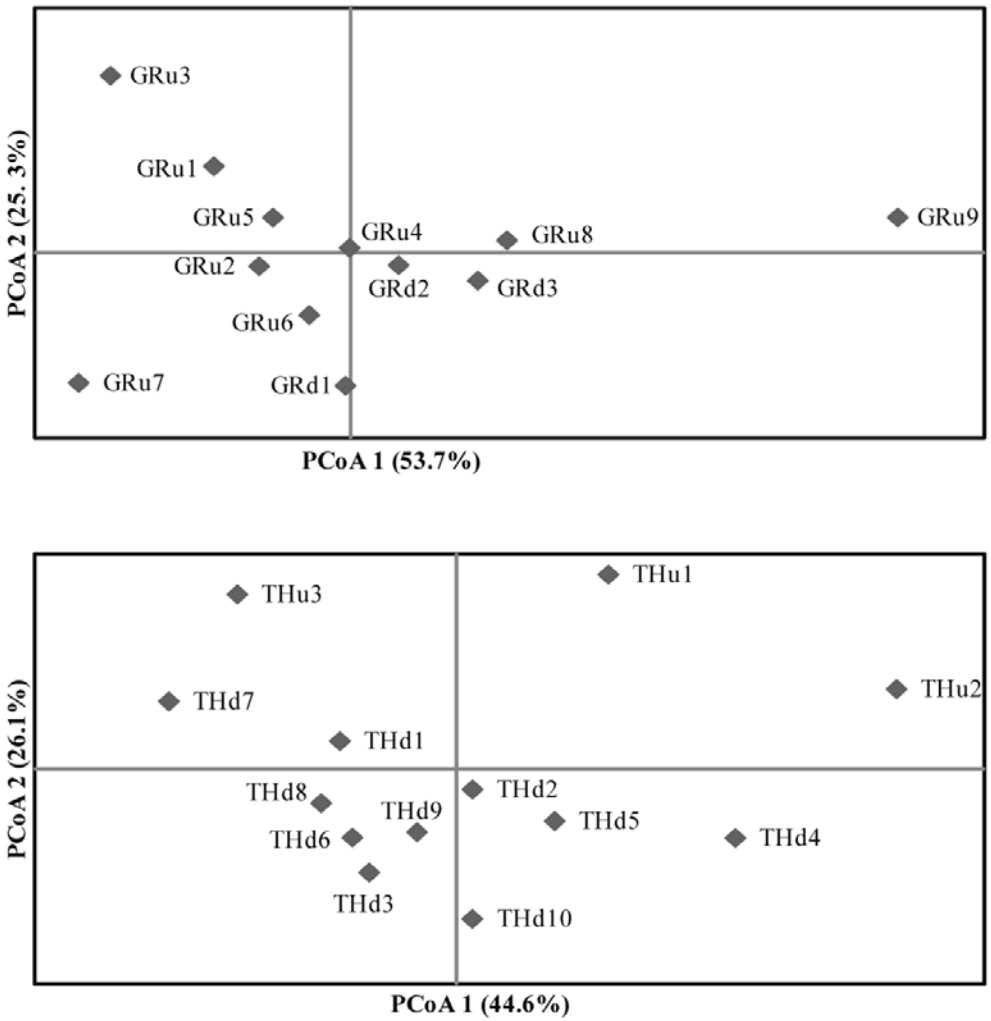

Figure 3.2: Principal coordinate analysis (PCoA) plot based on pairwise F_{ST} values among all sampled eastern sand darter sites for; A) Grand River and B) Thames River.

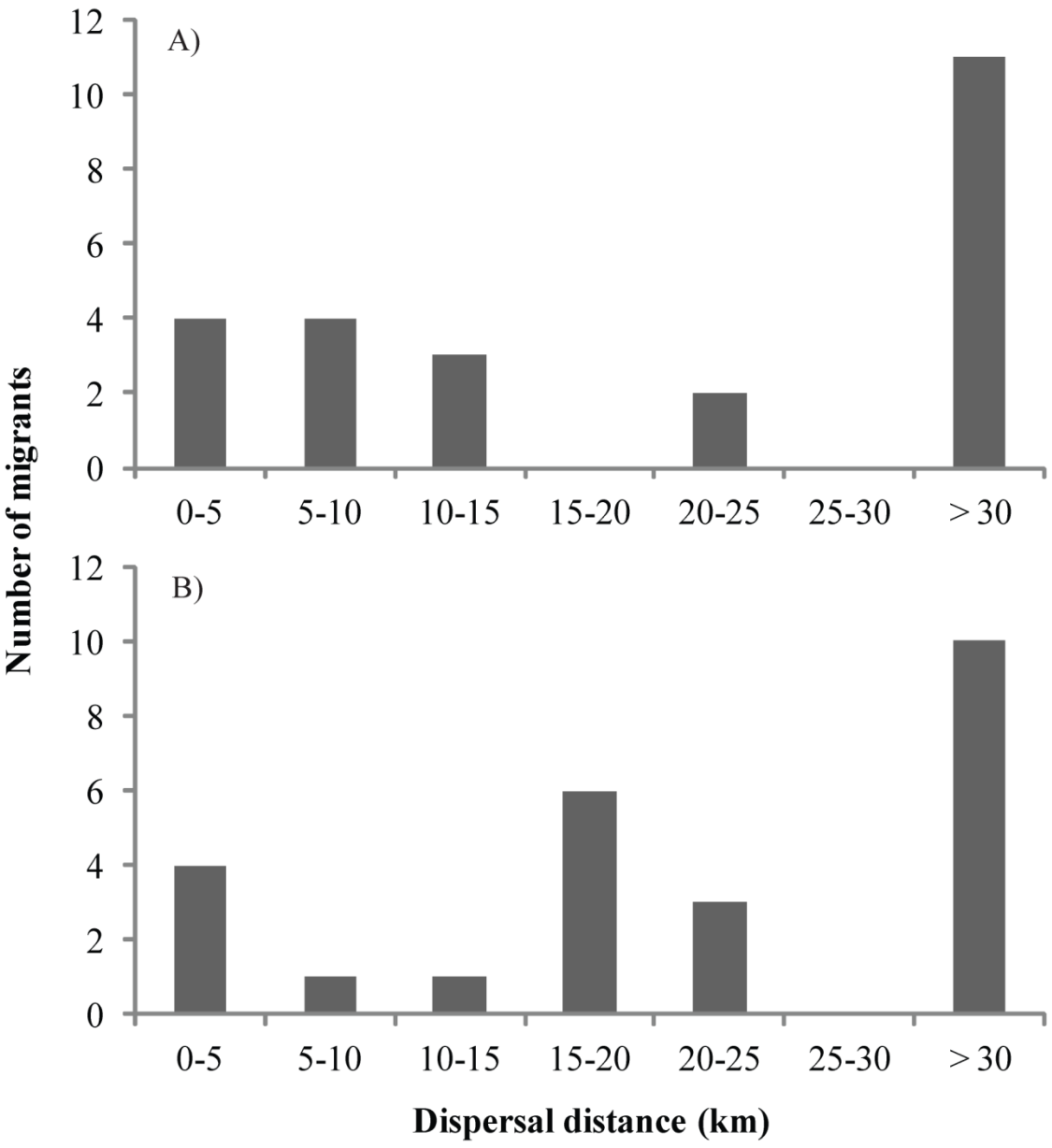

Figure 3.3: Frequency distribution of dispersal distances for eastern sand darters among sites within the: A) Thames River; and, B) Grand River, determined by genotype assignment. Dispersal distances were identified as the shortest hydrological distances separating two sites.

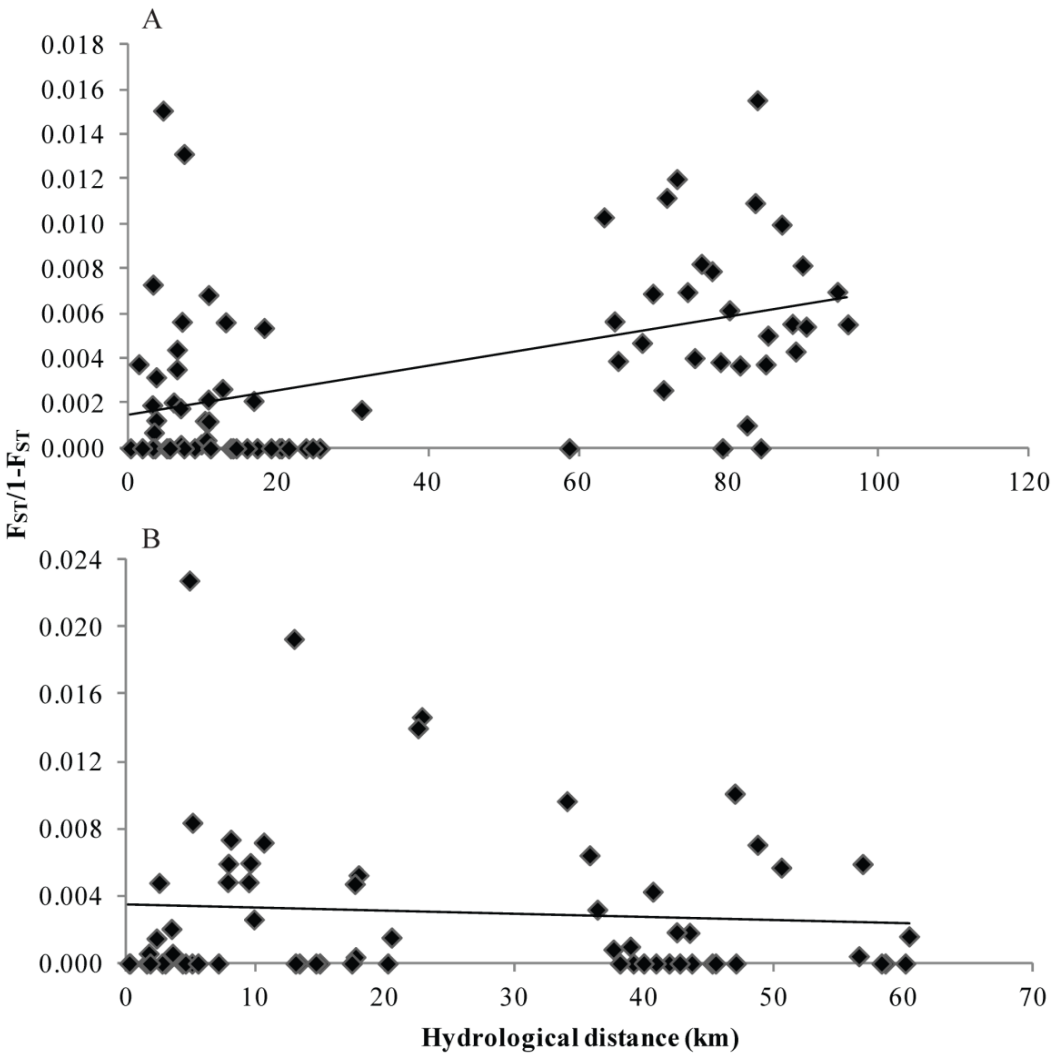

Figure 3.4: Relationship between linearized pairwise genetic differentiation ($F_{ST}/1-F_{ST}$) and hydrological distances (km) among eastern sand darter sample sites to test for isolation-by-distance (IBD) in: A) Thames River; and, B) Grand River. Mantel tests resulted in a strong correlation between loss of gene flow and increasing hydrological distances and a significant IBD relationship for the Thames River ($R^2 = 0.22$, $P = 0.012$), while the Grand River had no IBD correlation ($R^2 = 0.0065$, $P = 0.43$).

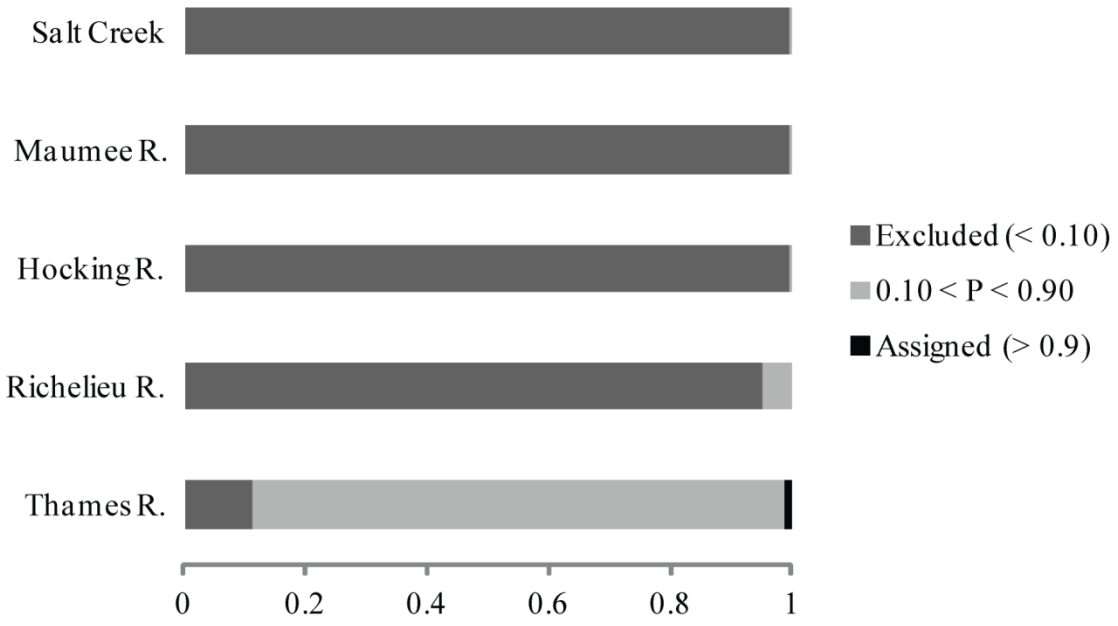

Figure 3.5: Genotype exclusion for the Grand River individuals collected in 2010 and the proportion of individuals that were excluded ($P < 0.10$), were uncategorized ($0.10 < P < 0.90$), and likely assigned ($P > 0.90$) to other eastern sand darter river populations (Salt Creek, Maumee River, Hocking River, Richelieu River, and Thames River; see Fig 2.1 for locations).

Appendix 3.1: Pairwise F_{ST} below diagonal and D_C values diagonal for all sample sites within the Thames and Grand rivers. For F_{ST} values, underline indicates significant differentiation at P = 0.05 as no values were significant following Bonferroni correction (P < 0.0001), while bold D_C values indicate significant exact tests (P < 0.05).

	THU1	THU2	THU3	THD1	THD2	THD3	THD4	THD5	THD6	THD7	THD8	THD9	THD10
THU1	*	0.261	**0.247**	**0.251**	**0.239**	**0.268**	**0.253**	**0.243**	**0.256**	**0.262**	**0.255**	**0.264**	**0.276**
THU2	0.004	*	**0.246**	**0.263**	**0.231**	**0.236**	**0.276**	**0.246**	**0.273**	**0.268**	**0.230**	**0.245**	**0.264**
THU3	0.002	0.015	*	0.209	0.221	0.224	**0.244**	0.241	0.247	0.231	0.195	0.215	**0.260**
THD1	0.006	0.010	-0.001	*	**0.238**	0.256	**0.260**	**0.251**	0.245	0.241	0.222	0.240	0.263
THD2	0.003	0.007	0.004	0.004	*	0.242	**0.236**	0.226	0.246	0.221	0.205	0.227	0.229
THD3	0.007	0.012	0.005	0.000	0.002	*	**0.256**	0.236	0.254	0.241	0.220	0.218	0.240
THD4	0.008	0.008	0.011	0.006	0.004	0.007	*	0.221	**0.250**	**0.270**	**0.235**	0.243	0.266
THD5	0.004	0.006	0.004	0.002	0.001	0.000	0.001	*	0.243	0.259	**0.234**	0.232	0.250
THD6	0.004	0.011	0.004	-0.001	-0.004	0.000	0.006	0.001	*	0.248	0.221	0.240	0.247
THD7	0.005	0.015	-0.004	-0.001	-0.002	0.001	0.013	0.003	-0.003	*	0.211	0.235	0.254
THD8	0.006	0.010	0.001	-0.001	0.000	0.000	0.007	0.002	-0.003	-0.003	*	0.197	0.235
THD9	0.005	0.004	-0.003	-0.002	-0.001	-0.003	0.003	-0.005	-0.004	-0.001	-0.007	*	0.231
THD10	0.006	0.007	0.008	0.002	-0.002	-0.003	0.005	-0.001	-0.005	0.002	-0.001	-0.007	*

	GRU1	GRU2	GRU3	GRU4	GRU5	GRU6	GRU7	GRU8	GRU9	GRD1	GRD2	GRD3
GRU1	*	0.189	**0.228**	0.210	0.239	0.205	0.240	0.192	0.256	**0.225**	0.214	0.193
GRU2	0.000	*	**0.245**	0.224	0.253	0.220	0.230	0.209	0.260	0.230	0.231	0.205
GRU3	0.003	0.006	*	0.232	0.292	**0.246**	**0.251**	**0.244**	**0.308**	**0.269**	**0.266**	**0.241**
GRU4	-0.002	-0.001	0.002	*	0.226	0.214	0.238	0.209	0.280	0.248	0.218	0.224
GRU5	-0.004	-0.008	-0.004	-0.007	*	0.247	0.251	0.232	0.289	0.268	0.263	0.251
GRU6	0.000	-0.003	0.005	-0.008	-0.005	*	0.239	0.224	0.270	0.248	0.242	0.201
GRU7	0.005	0.005	0.007	-0.001	-0.001	-0.002	*	0.234	**0.289**	0.246	0.254	0.229
GRU8	0.002	-0.002	0.007	-0.002	-0.004	-0.001	0.005	*	0.247	**0.236**	0.228	0.202
GRU9	0.014	0.014	0.019	0.005	0.006	0.008	0.022	0.001	*	0.283	0.261	0.243
GRD1	0.006	0.000	0.010	0.002	-0.003	-0.001	0.001	0.003	0.010	*	**0.262**	0.223
GRD2	-0.001	-0.001	0.007	-0.007	-0.005	-0.003	0.004	-0.002	0.006	0.001	*	0.232
GRD3	0.002	-0.001	0.006	-0.004	-0.003	-0.005	0.002	-0.003	0.001	0.001	-0.001	*

4.0 GENERAL DISCUSSION

In this thesis, I have demonstrated the value of analyzing populations at a variety of spatial scales to get an overall representation of the historic and contemporary processes that can shape population genetic structure. Identification of genetic structure patterns for eastern sand darter populations provides insight into how species dependent on specific habitats are able to compensate for habitat loss using unique dispersal techniques. Highlighted in this thesis are range-edge genetic effects in habitat-specific fish populations that contrast expectations and advocate the important influence that life-history characteristics have on shaping genetic structure. Genetic effects for northern range-edge populations revealed that unlike previously predictions, range-edge populations may not justify the increased conservation concerns for a species, especially when negative anthropogenic influences threaten populations throughout the entire range. I demonstrated strong historic influences on contemporary genetic structure which has persisted because of the isolating nature of large-flowing rivers or lake environments. I also use genetic analysis coupled with an understanding of the ecology of the eastern sand darter to identify genetically viable populations that can be used in the development of future translocation-based recovery strategies. I suggest, for the first time, an essential relationship between unstable habitat and elevated levels of within-river gene flow and this may be an important fundamental concept for genetic structure of other habitat-specific species. I further show in both studies that non-lethal sampling and molecular genetic markers can be used to quantitatively characterize population connectivity patterns that underlie range-edge effects and will prove valuable in the development of species recovery strategies.

My characterization of genetic diversity at multiple spatial-scales detected minimal gene flow among rivers, and showed that the range-wide genetic structure of eastern sand darter populations still reflects historic drainage connections that formed during the most recent Pleistocene glacial retreat. The historic Maumee connection has long-been expected to facilitate the colonization of the Great Lakes from the Mississippian Refugia and this is the only study to my knowledge that has provided genetic evidence of this using fish species. I also genetically verified a long-standing belief that Canadian eastern sand darter populations in Quebec and Ontario are demographically and genetically "disjunct" (COSEWIC 2011; Fisheries and Oceans Canada 2012). Furthermore, I showed that the historic separation of Quebec and Ontario populations resulted in heightened conservation concerns for the Quebec eastern sand darter populations and this is an important finding for other species that similarly colonized the St. Lawrence drainage following the Pleistocene glacial retreat.

Eastern sand darter are considered threatened in many rivers across their range, largely based on historic estimates of population size and the perceived loss of suitable habitats associated with the anthropogenic impacts. This is the first study to use genetic markers to determine eastern sand darter effective population sizes (N_E) and identify dispersal patterns within rivers, both of which are important to consider when developing conservation strategies (Armstong & Seddon 2007). This thesis revealed that both the Thames and Grand Rivers represent genetically viable populations and extensive gene flow among populations within the rivers acts to preserve genetic diversity. However, I demonstrate evidence that the Grand River eastern sand darter populations likely result from a recent introduction, perhaps driven by anthropogenic influences, thus the use of

the Grand River eastern sand darter in future re-introduction strategies should be avoided. I found that the Thames River contains eastern sand darter populations that are potentially critical for the future persistence of the species in southwestern Ontario, and perhaps Canada. Thames River populations may represent the only genetically, demographically, and geographically viable groups of populations that could be used in the reintroduction of eastern sand darter populations into extirpated habitat in southwestern Ontario.

4.1 FUTURE RESEARCH

This thesis has made substantial contributions to our knowledge of eastern sand darter population connectivity at different spatial scales. The extensive within-river gene flow had not been previously documented for eastern sand darter, while high genetic differentiation among rivers identifies apparent barriers to dispersal. Our study analyzed range-wide genetic structure using nuclear DNA (nDNA) microsatellite markers and these markers are effective in analyzing population connectivity. However, for a more accurate phylogenetic history of population genetic divergence across the species range we should further analyze populations using mitochondrial DNA markers as these are maternally inherited so they do not experience recombination as does nDNA and they experience higher mutation rates than nDNA (Lu *et al.* 2001; Cook *et al.* 2007). Having a more accurate phylogenentic resolution of population genetic divergence will allow us to explore population genetic divergence trends such as changes in river flows during river and landscape changes (e.g., isostatic rebound) that followed the Pleistocene glacial retreat and this will provide further resolution of the genetic similarities among some eastern sand darter sampled rivers.

There are important gaps in our ecological understanding of eastern sand darter that will limit the success of conservation actions. To build on the results of this thesis, a better understanding of many currently uninvestigated aspects of eastern sand darter biology, ecology, and life-history characteristics are needed for the proper implementation of the eastern sand darter recovery strategy (Fisheries and Oceans Canada 2012). Juveniles likely have a large influence on gene flow patterns as all of the juveniles examined in this study were migrants, most of which had a downstream directionality, so a better investigation into the juvenile life-stage will allow for resolution of a potential pelagic dispersal hypothesis for eastern sand darter. Also, the juvenile life-stage has been identified as the most important to conserve for future population viability; therefore, an understanding of specific juvenile habitat requirements will be especially beneficial for maintaining genetic connectivity in future recovery strategies (Finch 2009). Finally, eastern sand darter over-wintering habitats should be identified as these may provide an additional mechanism facilitating the unexpectedly high levels of within-river gene flow.

For conservation of eastern sand darter population in the southern range (i.e., Kentucky), that were not identified as genetically depauperate, conservation actions should focus on conserving suitable habitat to increase census size in the rivers that we sampled. For eastern sand darter populations in Quebec and Champlain Canal (New York) immediate development of genetic rescue strategies would be beneficial for restoration genetic diversity within these populations. The implementation of future recovery actions for eastern sand darter, including population reintroduction into extirpated areas and supplementation into drastically depleted regions, requires intensive

sampling of eastern sand darter preferred habitat to: a) ensure populations in the recipient river are actually extirpated; or, b) identify the genetic structure of recipient river populations to minimize the potential for outbreeding depression (Huff *et al*. 2010). For those rivers that have experienced population extirpation, the development and implementation of genetic rescue strategies are essential prior to reintroducing fish populations into those rivers and the implementation of a multi-disciplinary approach in determining the most appropriate source population will prove the most effective in conservation of eastern sand darter.

4.2 REFERENCES

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VITA AUCTORIS

NAME:	Robert James Ward Ginson
YEAR OF BIRTH:	1987
EDUCATION:	Blackville High School, Blackville, NB 2005- High School
	University of New Brunswick, Fredericton, NB 2005-2009 BSc. Biology
	Great Lakes Institute for Environmental Research University of Windsor, Windsor, ON 2010-2012, MSc. Environmental Science